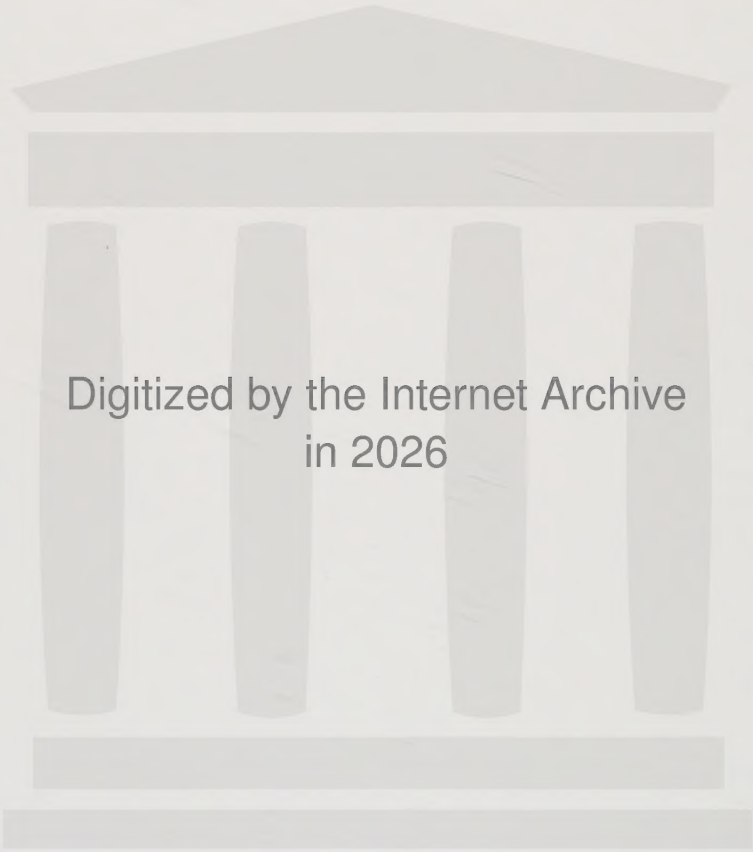
On the Peptidergic and Aminergic Regulation of Insulin Secretion

Bo Ahrén

Lund 1981


Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41553209_0004

On the Peptidergic and Aminergic Regulation of Insulin Secretion

Bo Ahrén

Department of Pharmacology
University of Lund

Lund 1981

UNIV.
LEIDEN
BIBL.

Printed in Sweden
Studentlitteratur
Lund 1981

Yahweh, you examine me and know me,
you know if I am standing or sitting,
you read my thoughts from far away,
whether I walk or lie down, you are watching,
you know every detail of my conduct.

The word is not even on my tongue,
Yahweh, before you know all about it;
close behind and close in front you fence me round,
shielding me with your hand.
Such knowledge is beyond my understanding,
a height to which my mind cannot attain.

Where could I go to escape your spirit?
Where could I flee from your presence?
If I climb the heavens, you are there,
there too, if I lie in Sheol.

If I flew to the point of sunrise,
or westward across the sea,
your hand would still be guiding me,
your right hand holding me.

If I asked darkness to cover me,
and light to become night around me,
the darkness would not be dark to you,
night would be as light as day.

It was you who created my inmost self,
and put me together in my mother's womb;
for all these mysterious I thank you:
for the wonder of myself, for the wonder of your works.

You know me through and through,
from having watched my bones take shape
when I was being formed in secret,
knitted together in the limbo of the womb.

You had scrutinised my every action,
all were recorded in your book,
my days listed and determined,
even before the first of them occurred.

God, examine me and know my heart,
probe me and know my thoughts;
make sure I do not follow pernicious ways,
and guide me in the way that is everlasting.

Of David

The dissertation is based on the following papers which in the text will be referred to by their Arabic numerals:

1. B. Ahrén, I. Lundquist: Effects of two cholecystokinin variants, CCK-39 and CCK-8, on basal and stimulated insulin secretion. Submitted.

2. B. Ahrén, I. Lundquist: Effects of vasoactive intestinal polypeptide (VIP), secretin, and gastrin on insulin secretion in the mouse. Diabetologia 20:1-6 (1981)

3. B. Ahrén, R. Håkanson, I. Lundquist, K. Sjölund, F. Sundler: GIP-like immunoreactivity in glucagon cells. Interactions between GIP and glucagon on insulin release. Acta Physiol Scand, in press (1981)

4. B. Ahrén, I. Lundquist: Effects of glicentine on insulin secretion. Horm Metab Res 12:582-586 (1980)

5. I. Lundquist, F. Sundler, B. Ahrén, J. Alumets, R. Håkanson: Somatostatin, pancreatic polypeptide, substance P, and neurotensin: Cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology 104:832-838 (1979)

6. B. Ahrén, L.E. Ericson, I. Lundquist, I. Lorén, F. Sundler: Adrenergic innervation of pancreatic islets and modulation of insulin secretion by the sympatho-adrenal system. Cell Tiss Res, in press (1981)

7. B. Ahrén, I. Lundquist: Adrenalectomy and chemical sympathectomy by 6-hydroxydopamine: Effects on basal and stimulated insulin secretion. Pflügers Arch, in press (1981)

8. B. Ahrén, I. Lundquist: Effects of selective and nonselective β-adrenergic agents on insulin secretion in vivo. Europ J Pharmacol, in press (1981)

9. B. Ahrén, I. Lundquist: Effects of autonomic blockade by methylatropine and optical isomers of propranolol on plasma insulin levels in the basal state and after stimulation. Acta Physiol Scand, in press (1981)

10. B. Ahrén, J. Järhult, I. Lundquist: Influence of the sympatho-adrenal system and somatostatin on the secretion of insulin in the rat. J Physiol, in press (1981)

11. B. Ahrén, J. Järhult, I. Lundquist: Insulin secretion induced by glucose and by stimulation of β_2-adrenoceptors. Different sensitivity to somatostatin. Submitted.

12. I. Lundquist, B. Ahrén, R. Håkanson, F. Sundler: The role of intracellular amines in the regulation of islet cell function. In: W.K. Waldhäusl (ed) Diabetes 1979, Excerpta Medica, Amsterdam, Oxford, Princeton, pp. 57-63 (1980)

13. B. Ahrén, I. Lundquist: Insulin secretory response to different secretagogues in hyper- and hypothyroid mice. Submitted.

These papers are summarized and discussed together with some new and unpublished information. This summary was completed November 28th, 1980.

CONTENTS

		Page
I.	INTRODUCTION	9
II.	METHODOLOGICAL CONSIDERATIONS	11
III.	PEPTIDERGIC INFLUENCES ON INSULIN SECRETION	15
	A. General Considerations	15
	1. Gastrointestinal Hormones	15
	2. Islet Polypeptides	17
	3. Neuropeptides	18
	B. Present Results	18
	1. Cholecystokinin	18
	2. Gastrin	21
	3. Secretin	22
	4. Pancreatic Glucagon	23
	5. Extrapancreatic Glucagon, Glicentin	25
	6. GIP	25
	7. VIP	26
	8. Somatostatin	27
	9. PP	30
	10. Substance P	31
	11. Neurotensin	32
	12. Summary	33
IV.	INFLUENCES OF THE AUTONOMIC NERVOUS SYSTEM ON INSULIN SECRETION	35
	A. General Considerations	35
	1. The Cholinergic Nervous System and Insulin Secretion	35
	2. The Adrenergic Nervous System and Insulin Secretion	35
	B. Present Results	36
	1. The Sympatho-Adrenal System and Basal Insulin Secretion	36
	2. The Sympatho-Adrenal System and Stimulated Insulin Secretion	37
	3. The β-Adrenoceptor and Insulin Secretion	38
	4. The Muscarinic Receptor and Insulin Secretion	40
	5. Summary	41
V.	INFLUENCES OF INTRACELLULAR DOPAMINE AND THE THYROID STATE ON INSULIN SECRETION	45
	1. Intracellular Dopamine Accumulation	45
	2. Hyper- and Hypothyroidism	47
	3. Summary	48
VI.	SUMMARY AND CONCLUSIONS	49
VII.	ACKNOWLEDGEMENTS	51
VIII.	REFERENCES	52
IX.	APPENDIX: Papers 1-13.	69

ABBREVIATIONS

ABTS	2,2'-azino-di-(3-ethyl-benzthiazoline-(6)-sulfonate)
APP	Avian pancreatic polypeptide
BPP	Bovine pancreatic polypeptide
cAMP	Cyclic adenosine 3'5'-monophosphate
cGMP	Cyclic guanosine 3'5'-monophosphate
CCK	Cholecystokinin (CCK-8 denotes the C-terminal octapeptide of CCK, CCK-33 tricontatriapeptide and CCK-39 tricontanonapeptide cholecystokinin)
C-peptide	Connecting peptide
DOPA	3,4-Dihydroxyphenylalanine
Gastrin-4	Tetrapeptide gastrin
Gastrin-17	Heptadecapeptide gastrin
Gastrin-34	Tricontatetrapeptide gastrin
GIP	Gastric inhibitory polypeptide or Glucose-dependent insulinotrophic polypeptide
GLI	Glucagon-like immunoreactivity
5-HTP	5-Hydroxytryptophan
ICI 118,551	Erythro-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol
IPNA	Isopropylnoradrenaline (isoprenaline, isoproterenol)
ITP	1-Isopropylamino-3-(2-thiazoloxy)-2-propanol
PP	Pancreatic polypeptide
VIP	Vasoactive intestinal polypeptide

The prefixes L- and D- (e.g. L-propranolol, D-propranolol) denote the levo- and dextro-rotatory isomers of the substances, respectively.

I. INTRODUCTION

The concentration of plasma glucose is under the control of several humoral and neural mechanisms (cf. Ensinck and Williams 1974). One of the main regulatory principles is insulin. Insulin lowers plasma glucose through enhancement of glucose uptake in various tissues and through inhibition of glucose delivery by the liver (cf. Cahill 1971). Plasma insulin concentration is regulated mainly by changes in liver extraction of insulin and by insulin secretion from the pancreatic islets, rather than by changes in the rate of peripheral uptake, degradation and elimination of insulin (Cahill 1976). Thus, knowledge of the regulation of insulin secretion is necessary for an understanding of glucose homeostasis in health and disease. Disturbances in the secretory capacity of the insulin cells may impair glucose tolerance and perhaps lead to diabetes mellitus (cf. Cerasi and Luft 1967). The pathogenesis of diabetes is, however, extremely complex and involve, in addition, changes in insulin sensitivity and plasma levels of other glucoregulatory factors (cf. Sherwin and Felig 1978).

An important role of the pancreas in the regulation of glucose homeostasis was suggested by Minkowski (1893). Later this concept was developed further by the isolation of insulin from the pancreas (Banting and Best 1922). Insulin is a polypeptide consisting of 51 amino acids arranged in two chains joined by two disulfide bridges. It is formed from proinsulin, after cleavage to insulin and C-peptide (cf. Steiner 1969).

Insulin and C-peptide are stored together in the secretory granules and are released in equimolar amounts into the circulation upon stimulation of the insulin cells (Rubinstein et al. 1969). The main regulatory factor for the secretion is thought to be glucose. According to existing evidence, glucose induces insulin secretion through complex ionic and metabolic events. Glucose is also thought to induce an increase in formation of cAMP, which potentiates glucose-induced insulin secretion. The final release of insulin is believed to involve microfilamentous-microtubular proteins leading to exocytosis of the secretory granules. Here Ca^{++} is of critical importance and certain intracellular Ca^{++} pools regulate the final steps in the secretion (for recent reviews on this complicated topic see Matthews 1977, Hellman et al. 1979, and Hedeskov 1980).

Several other physiological factors than glucose are also involved in the precise regulation of insulin secretion. Such factors are adrenergic, cholinergic and peptidergic nerves, hormones secreted by other endocrine cells, nutrients other than glucose, and secretory messengers such as cAMP, Ca^{++} and prostaglandins (Woods and Porte 1974, Unger et al. 1978, Creutzfeldt 1979, Robertson 1979, Hedeskov 1980). The mechanisms by which secretagogues other than glucose act are less well characterized, although intracellular metabolism, ionic fluxes and cyclic nucleotides have been shown to be altered by a variety of such stimuli (Kuo et al. 1973, Burr et al. 1976, Grodsky et al. 1977, Gagerman et al. 1978, Hedeskov 1980). Most evidence has been obtained from studies on isolated islets. However, insulin secretion *in vivo* depends on integrated functions of several factors. Thus, *in vivo* experiments are important for the understanding of the physiological regulation of insulin secretion.

The purpose of this summary is to present a study dealing with the peptidergic and aminergic regulation of insulin secretion *in vivo*. More precisely, the aim of the present work was to study how basal and stimulated insulin secretion is affected by:

a) islet hormones, gut hormones and neuropeptides
b) chemical sympathectomy, surgical splanchnicotomy, and adrenalectomy
c) β-adrenoceptor and muscarinic receptor blockade
d) intracellular dopamine accumulation
e) hyper- and hypothyroidism

II. METHODOLOGICAL CONSIDERATIONS

Animals

Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25-35 g and female rats of the Wistar strain (Møllegaard Avlslaboratorium, Skensved, Denmark), weighing 250-350 g, were used. In one series of experiments, pigmented guinea pigs of both sexes (Anticimex, Stockholm, Sweden), weighing 350-450 g, were used. All animals were fed a standard pellet diet (Astra-Ewos, Södertälje, Sweden) with tap water ad libitum. Adrenalectomized mice (papers 6 and 7) were kept on the standard pellet diet and given 0.154 mol/l saline to drink after the operation.

Insulin Secretory Experiments in Mice

In experiments reported in papers 1-9 and 12-13, various substances were injected intravenously into mice. Blood samples (100-250 µl) were taken by orbital puncture. It has earlier been shown that the orbital puncture technique is suitable when sampling of mouse plasma is desirable (Rerup and Lundquist 1966, Lundquist 1971). To avoid interexperimental differences in plasma levels of glucose and insulin, all individual experiments included saline-injected controls. All experimental values are related to the control values in the same experiments.

The experiments were performed on conscious and non-fasted mice. This exclude influences by anaesthesia and fasting on insulin secretory responses to stimulation (Feldman and Lebovitz 1973, Trimble et al. 1980).

In different experiments, the animals were subjected to chemical sympathectomy and adrenalectomy, or pretreated with autonomic blockade. Some animals were pretreated with L-DOPA for inducing an intracellular accumulation of dopamine. In other animals, hyper- or hypothyroidism was induced.

a) Chemical sympathectomy was induced by a single intravenous injection of 6-hydroxydopamine (40 mg/kg). 6-Hydroxydopamine resulted in a considerable reduction in the number of adrenergic nerve fibers in the pancreatic islets, and caused a severe damage of the nerve terminals. Islet endocrine cells seemed unaffected (papers 6 and 7).

b) Adrenalectomy was performed under light ether anaesthesia by the lumbar approach. In some of these animals, chemical sympathectomy by 6-hydroxydopamine was also performed (papers 6 and 7).

c) Autonomic blockade was performed by intraperitoneal injection of various drugs, nonselective as well as selective β-adrenoceptor blockers, and the muscarinic blocker methylatropine (papers 1, 8 and 9).

d) For inducing an intracellular accumulation of the monoamine dopamine, the animals were treated with the monoamine oxidase inhibitor pargyline and the amino acid L-DOPA. L-DOPA (0.26 mmol/kg) was given 2 hours prior to the experiments, and pargyline 2 hours (0.26 mmol/kg) or additionally 18 hours (0.52 mmol/kg) prior to L-DOPA-administration. It was necessary to inject the amino acid and not the amine, since the monoamine itself was found to be taken up poorly by the insulin cells in vivo. In one series of experiments, islets were isolated after collagenase digestion from mice pretreated with L-^{14}C-DOPA or ^{14}C-dopamine, in order to investigate the cellular uptake of L-DOPA and dopamine. In a separate series of experiments, the cellular uptake of the amino acid L-5-HTP and the amine serotonin was studied (paper 12).

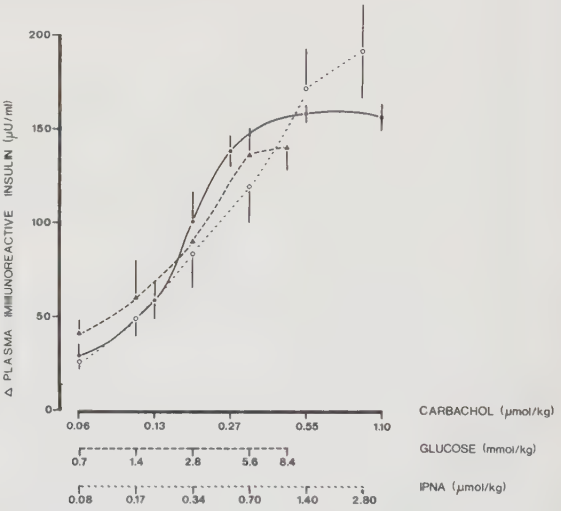
e) Hyperthyroidism was induced by daily subcutaneous injections of L-triiodothyronine (5 µg/mouse) for three weeks, and hypothyroidism by a single

intraperitoneal injection of ^{131}I (200 μCi/mouse) (paper 13).

The effects on basal insulin secretion of glucose, various polypeptides, β-adrenoceptor agonists, the α-adrenoceptor blocker phentolamine, and the cholinergic agonist carbachol, were initially characterized with regard to the dose-response and time-course. Figure I shows the dose-response curves for the effects of glucose, the nonselective β-adrenoceptor agonist L-IPNA, and carbachol, on insulin secretion. Corresponding curves for the β_1-adrenoceptor agonist ITP and the β_2-adrenoceptor agonist terbutaline may be seen in paper 8, and for various polypeptides in papers 1, 2, and 3.

Fig. I

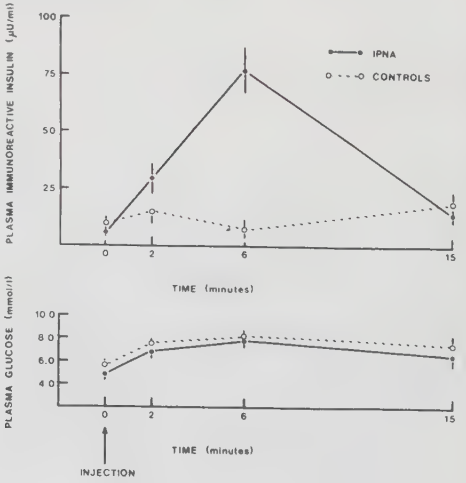
Dose-response relationships between dose of carbachol (μmol/kg), D-glucose (mmol/kg) and L-IPNA (μmol/kg), and acute insulin secretion in mice. Plasma concentrations of immunoreactive insulin were measured in samples taken 2 min (carbachol and D-glucose) and 5.5 min (L-IPNA) after the i.v. injection. Mean ± SEM is shown. Each group consisted of 8-16 animals.



The time-course of the insulin-releasing effect of L-IPNA may be seen in Fig. II, of glucose, carbachol, and terbutaline in paper 8, of phentolamine in paper 6, and of various polypeptides in Fig. III.

Fig. II

Changes in plasma concentrations of immunoreactive insulin and glucose following an i.v. injection of L-IPNA (0.34 μmol/kg) and saline, respectively, in mice. Mean ± SEM is shown. Each group consisted of 12 animals.



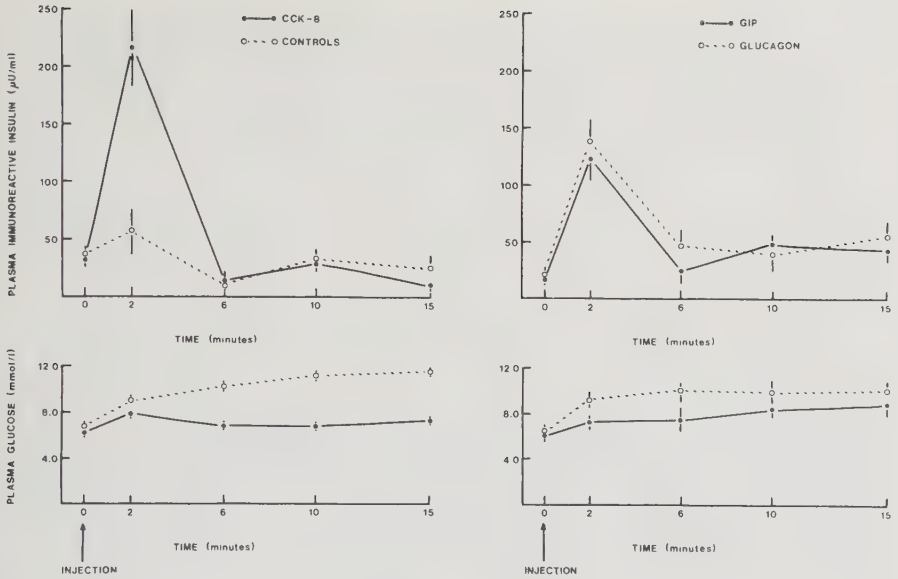


Fig. III. Changes in plasma concentrations of immunoreactive insulin and glucose following an i.v. injection of CCK-8 (6.25 nmol/kg), glucagon (4.25 nmol/kg), GIP (2.13 nmol/kg), and saline, respectively, in mice. Mean ± SEM is shown. Each group consisted of 10 animals.

Subsequently, the influences of various polypeptides, chemical sympathectomy, adrenalectomy, autonomic blockade, intracellular dopamine accumulation, and changes in the thyroid state on stimulated insulin secretion were investigated. In most experiments, four different secretagogues, which probably act through different mechanisms, were used as stimulators of insulin secretion: 1) glucose, 2) carbachol, 3) L-IPNA or terbutaline, and 4) CCK-8.

Insulin Secretory Experiments in Rats

In vivo. Intact rats or rats pretreated with surgical splanchnicotomy, chemical sympathectomy or adrenalectomy, were used (papers 6, 7, 10, and 11). Splanchnicotomy was performed under light ether anaesthesia by cutting the splanchnic nerves just beneath the diaphragm. Chemical sympathectomy was induced by an i.v. injection of 6-hydroxydopamine (65 mg/kg). All infusion experiments (papers 10 and 11) were performed under pentobarbitone anaesthesia. Various drugs were infused into a femoral vein and blood sampling was made from a femoral artery. Adrenalectomy was performed immediately prior to experiments reported in paper 10.

In a long-term study, blood samples in the conscious rat were taken by orbital puncture (paper 6). The intravenous glucose tolerance test (paper 7) was performed in conscious rats.

In vitro. The influence of intracellular dopamine accumulation on $^{45}Ca^{++}$--efflux was studied in a perifusion system using isolated rat islets (Frankel et al. 1978).

Morphological Investigations

In collaboration with Dr. Rolf Håkanson, Dept. Pharmacology, Lund, and

Dr. Frank Sundler, Dept. Histology, Lund, an immunohistochemical demonstration of various gut and islet peptides according to the methods of Coons et al. (1955) and Sternberger (1974), a demonstration of formaldehyde-induced fluorescence in islets of guinea pigs and mice according to Falck and Hillarp (1962), and microspectrofluorometry of guinea pig islets (Lundquist et al. 1975) were performed (papers 3, 5, 6, and 12).

Furthermore, in collaboration with Dr. Ingemar Lorén, Dept. Histology, Lund, the adrenergic nerves in the pancreas were demonstrated by the aluminium-formaldehyde histofluorescence technique according to Ajelis et al. (1979) (paper 6). In collaboration with Dr. Lars E Ericson, Dept. Anatomy, Göteborg, islets from normal and chemically sympathectomized mice were studied electron microscopically (paper 6).

More detailed descriptions of the various methods, and the sources of the different chemicals and drugs, may be seen in the material sections of the various papers.

Determination of Insulin, Glucagon, Glucose, and Glycogen

Plasma concentrations of immunoreactive insulin and glucagon were determined radioimmunochemically (Heding 1966, Heding 1971) using ^{125}I-labelled porcine insulin and glucagon and guinea pig anti-porcine insulin and rabbit anti-porcine glucagon. The glucagon antiserum (K 964) is specific for pancreatic glucagon (Novo Res Inst, Copenhagen, Denmark).

Plasma glucose concentrations were determined by the glucose oxidase method of Marks (1959), with slight modifications. The total volume of the deproteinizing fluid (NaOH + $ZnSO_4$ in NaCl) was 2 ml, the plasma samples were 10 µl, and ABTS was used as reagent (Bruss and Black 1978).

Tissue (liver and muscle) glycogen concentrations were determined in one series of experiments (paper 13) by the method of Rerup and Lundquist (1967).

III. PEPTIDERGIC INFLUENCES ON INSULIN SECRETION

In this chapter some basic knowledge of the polypeptides thought to be involved in the regulation of insulin secretion is initially summarized. Afterwards, the effects on insulin secretion of various polypeptides investigated in the present study are presented.

A. General Considerations

Polypeptides may influence insulin secretion through endocrine, paracrine and neurocrine actions.

1. Gastrointestinal Hormones

The first hormone discovered in the gastrointestinal tract was secretin (Bayliss and Starling 1902). Since then, a great and still increasing number of gastrointestinal endocrine cells and putative hormones have been identified. New informations of these cells and peptides evolve rapidly, and it is now established that the endocrine cell mass of the gut mucosa exceeds that of any other endocrine organ in the body (for recent reviews see Pearse et al. 1977, Grube and Forssmann 1979, and Larsson 1980).

The different gastro-entero endocrine cells are situated along the surface of the gastrointestinal tract. They have been distinguished by various light and electron microscopic methods. Further, by immunocytochemistry the cell types have been associated with certain polypeptide immunoreactivity. In some instances, a certain cell type reacts immunologically with two or more different antisera. Our present knowledge concerning the different gastro-entero-pancreatic endocrine cells and their assumed polypeptide content is summarized in Table I.

Table I

The various endocrine gastro-entero-pancreatic cells according to conventional nomenclature, with their assumed polypeptide content.

Cell Type	Polypeptides	Localization
G cells	Gastrin	gastric antrum duodenum
I cells	CCK	small intestine
S cells	Secretin	small intestine
A cells	Glucagon Glicentin	islets stomach (dog)
L cells	Glicentin Glucagon-like	intestines
D cells	Somatostatin	islets stomach intestines
PP cells	PP	islets stomach small intestine (few species)
N cells	Neurotensin	ileum
EC_1 cells	Substance P	intestines
K cells	GIP	small intestine
Mo cells	Motilin	small intestine
B cells	Insulin	islets

The amino acid sequences of some of the gastrointestinal polypeptides have been established, and it has been documented, that sequence similarities between certain polypeptides occur (Dockray 1977). At least two main polypeptide families have been suggested to exist: the gastrin family, including gastrin and CCK, and the secretin family, including secretin, glucagon, VIP, and GIP, respectively. The close structural similarities between the polypeptides in the same family might account for similarities in function. Several polypeptides, such as somatostatin, substance P, and neurotensin, share no obvious structural similarity with those belonging to the gastrin and the secretin family. Table II shows the amino acid sequences of the polypeptides more closely investigated in the present study.

Table II

Amino acid sequences of porcine CCK-39, gastrin-17, secretin, glucagon, GIP, VIP, and of bovine somatostatin, substance P, neurotensin, and PP.

	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
CCK	Tyr-Ile-Gln-Gln-Ala-Arg-Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-Ile-Lys-Asn-Leu-Gln-Ser-Leu-
Gastrin	Glu-Gly-Pro-Tyr-Met-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe
Secretin	His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-Ser-Ala-Arg-Leu-Gln-Arg-Leu-
Glucagon	His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-
GIP	Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-Arg-Gln-Gln-Asp-Phe-
VIP	His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-
Somatostatin	Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys
BPP	Ala-Pro-Leu-Glu-Pro-Gln-Tyr-Pro-Gly-Asp-Asp-Ala-Thr-Pro-Glu-Gln-Met-Ala-Gln-Tyr-Ala-Ala-
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met
Neurotensin	Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu

	23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43
CCK	-Asp-Pro-Ser-His-Arg-Ile-Ser-Asp-Arg-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe
Secretin	-Leu-Gln-Gly-Leu-Val
Glucagon	-Val-Gln-Trp-Leu-Met-Asn-Thr
GIP	-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys-Gly-Lys-Lys-Ser-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln
VIP	-Leu-Asn-Ser-Ile-Leu-Asn
BPP	-Glu-Leu-Arg-Arg-Tyr-Ile-Asn-Met-Leu-Thr-Arg-Pro-Arg-Tyr

The gastrointestinal polypeptide hormones are assumed to be stored in the secretory granules of the cells. Upon stimulation, the granules are extruded by exocytotic mechanisms and their content is liberated extracellularly. The polypeptides may then influence the activity of adjacent cells (paracrine influences). In addition, the polypeptides may enter the blood stream and influence distant situated target organs, e.g. the pancreas (endocrine influences). Direct release of gastrointestinal polypeptides into the lumen of the digestive tract has also been demonstrated (Uvnäs-Wallensten 1980, Wisén et al. 1980), but the relevance of this intraluminal secretion is at present not established.

Many gastrointestinal hormones are secreted into the blood after feeding (Hornnes et al. 1980). GIP is released after oral intake of carbohydrate, protein and fat. The secretion of secretin, gastrin and CCK is most potently stimulated by a protein-rich meal (cf. Creutzfeldt 1979).

Shortly after the discovery of secretin, it was suggested that this polypeptide might influence the endocrine pancreas in analogy with its influence on the exocrine pancreas (Moore et al. 1906). About 20 years later it was found that a crude preparation of secretin induced hypoglycemia when injected intravenously into dogs; this effect was presumed to be mediated by an enhanced insulin secretion (LaBarre and Still 1930).

This activity, potentiation of glucose-induced insulin secretion by gastro-intestinal hormones, has since been called "incretin activity" (cf. Creutzfeldt 1979).

In 1964 it was demonstrated that oral ingestion of glucose induced a much greater increase in plasma insulin concentrations than intravenous injection of a similar amount of glucose, despite a smaller elevation of plasma glucose levels (McIntyre et al. 1964, Elrick et al. 1964). Later it was calculated that at least half of the secreted insulin after oral glucose intake could be ascribed to gastro-intestinal factors potentiating the glucose-induced insulin release (Perley and Kipnis 1967).

Several studies have been undertaken in order to establish whether one or several of the gastrointestinal hormones potentiate glucose-induced insulin secretion. It has been found that gastrin-4 (Rehfeld 1971), secretin (Unger et al. 1967, Lerner 1977), CCK-8 (Frame et al. 1975), GIP (Pederson et al. 1975) and VIP (Ohneda et al. 1977, Kaneto et al. 1977) can stimulate insulin secretion in vivo in different species. Most results available indicate that GIP is the main incretin candidate (Creutzfeldt 1979).

Results obtained during the few last years have made the incretin concept more complicated. Thus, several new hormone candidates of peptide nature have been discovered in the gut. Furthermore, a wide chemical heterogeneity of certain of the polypeptides has been demonstrated. Consequently, the number of possible incretin candidates has increased considerably. Also, the relation between the release of the polypeptides after meal ingestion and insulin secretion is not established. In addition, oral ingestion of liquid, without any nutrients, potentiates the insulin secretory response to a concomitant glucose infusion (Goldberg et al. 1980). This shows that ingestion of nutrients is not a necessity for inducing a potentiated insulin response to glucose by the gut.

Recent experimental studies have shown that other factors than GIP are involved in the classical incretin effect. Thus, intravenous administration of antiserum against pure GIP did not abolish the incretin effect after fat ingestion (Ebert et al. 1979). Moreover, effects on insulin secretion of portal venous effluent from the glucose-perfused rat gastrointestinal tract, have been shown to be clearly different from the effects of several known gastrointestinal polypeptides, including GIP (Levin et al. 1979).

2. Islet Polypeptides

The pancreatic islets were first described by Paul Langerhans (1869). It has been demonstrated in different laboratories that the islets of Langerhans consist of several cell types producing different polypeptides such as insulin, glucagon, somatostatin, and pancreatic polypeptide (Baum et al. 1962, Luft et al. 1974, Dubois 1975, Unger and Orci 1976, Larsson et al. 1976a, Unger et al. 1978). In most species, insulin cells comprise the central part of the islets. Peripherally located are glucagon cells, PP cells, and somatostatin cells (cf. Unger et al. 1978). Regional differences in the pancreas exist, and PP cells are more numerous in the duodenal part, whereas the reverse is the case for glucagon cells (Larsson et al. 1976a).

The topographical arrangement of the islets suggests, that an intra-islet paracrine regulatory system might exist with regard to polypeptide secretion. Indeed, it has been shown that glucagon has the ability to enhance, and somatostatin to depress insulin secretion, whereas insulin can inhibit the secretion of glucagon (Samols et al. 1965, Koerker et al. 1974, Mortimer et al. 1974, Östenson 1979). It is also notable that antisera against insulin in vivo or somatostatin in vitro has been reported to in-

crease the secretion of glucagon or insulin (Müller et al. 1971, Barden et al. 1977, Taniguchi et al. 1977). Such paracrine influences can be promoted by intercellular junctions (Staehelin 1974).

By electron microscopy it has been demonstrated that 'tight junctions' exist in the islets (Orci et al. 1973, Orci 1976). Tight junctions are structures at the cell membranes where adjacent cells are adjoining. These tight junctions have the capability to compartmentalize intercellular spaces (cf. Orci 1976). Hence, the possibility of high concentrations of a secretory product of a cell in local spaces exists.

In addition, adjacent cells may influence each other through 'gap junctions'. These are small gaps in the cell membranes of two adjacent cells, through which small molecules can pass from one cell to another without extracellular passage of the substances. Gap junctions have been demonstrated in the islets (Orci et al. 1973). Polypeptides can probably not pass through a gap junction, but second messengers, such as cAMP, may do it (Lawrence 1978). In islets, ions (Meissner et al. 1976) and small molecules, such as uridine nucleotides (Meda et al. 1980), probably have the capability to pass from one insulin cell to another. The development of both tight and gap junctions seems to be regulated by extrinsic factors, e.g. glucose (Orci 1976, Meda et al. 1980).

3. Neuropeptides

A great number of biologically active polypeptides, such as opioid peptides, substance P, neurotensin, VIP, CCK-8, and somatostatin, have during recent years been shown to occur in neurons, with or without a concomitant occurrence in endocrine cells. The neuropeptides are assumed to function as neurotransmitters; this seems to be established for a few (Snyder and Innis 1979). In the gut, the peptidergic nervous system is suggested to be of physiological importance, integrating extrinsic and intrinsic influences at the local level (Sundler et al. 1980).

In the pancreas, neurons containing material reacting with antisera against VIP, substance P, enkephalin, and gastrin/CCK have been described, and a peptidergic innervation of the pancreatic islets by gastrin/CCK- and VIP-immunoreactive nerves has been demonstrated (Larsson 1979, Rehfeld et al. 1980, Bishop et al. 1980). Whether influences of neuropeptides on insulin secretion are of physiological importance is still uncertain.

B. Present Results

One of the aims of the present investigation was to compare the *in vivo* effects of various polypeptides on basal and stimulated insulin secretion under identical conditions in the mouse. Thus, the effects of the polypeptides on basal insulin secretion were studied, and the influences of them on insulin secretion stimulated by glucose, the cholinergic agonist carbachol and the ß-adrenoceptor agonist L-IPNA were investigated. In these latter experiments, doses of the polypeptides which were without demonstrable effects on basal insulin secretion were used. The influences of autonomic blockade, the sympatho-adrenal system, intracellular dopamine, and thyroid activity on the effects of some polypeptides were also studied. In addition, the cellular distribution of some of the polypeptides were mapped by immunohistochemical methods.

1. Cholecystokinin

CCK was originally isolated from the duodenal mucosa and described as a

factor that induced contraction of the gallbladder (Ivy and Oldberg 1928). Later, a factor, originally named pancreozymin, was also isolated from duodenal mucosa and shown to stimulate pancreatic enzyme secretion (Harper and Raper 1943). Subsequently it was found that these two substances were identical (cf. Mutt 1979). Immunocytochemistry has revealed CCK cells in the mucosa of the small intestine (Polak et al. 1975). A suggestion that CCK occurs in glucagon cells in the pancreas has been forwarded in one study (Grube et al. 1978) but it is not supported by others (Polak et al. 1975).

CCK was initially described as a 33 amino acid polypeptide but later studies revealed that a variant with 39 amino acids and even more variants, such as CCK-8, may exist (Mutt 1979). It has been suggested that the biological activity of different forms of CCK is contained in the C-terminal octapeptide, CCK-8 (Ondetti et al. 1970). Thus, CCK-33 and CCK-39 may be prohormones since these two polypeptides may be degraded to CCK-8 (cf. Mutt 1979).

Beside the localization to endocrine cells in the gut, CCK-immunoreactivity has been localized to central and peripheral neurons (Rehfeld 1978, Larsson and Rehfeld 1979, Rehfeld et al. 1980); the latter found in gut and pancreas. It has been shown in studies on human and porcine tissues, that the most abundant form of the CCK immunoreactivity in the gut and brain is due to CCK-8; only a small per cent is CCK-33 or CCK-39 (Rehfeld 1978). Recently, gastrin/CCK-nerves were located in the pancreas and found to innervate pancreatic islets; they seem to contain mainly CCK-8 and gastrin-4 (= CCK-4) (Rehfeld et al. 1980). Thus, CCK may influence insulin secretion by endocrine and neurocrine modes of action.

The effect of CCK-33 on insulin secretion has been investigated in several studies, and a stimulatory effect has been demonstrated in vivo in man (Raptis et al. 1975), in dogs (Unger et al. 1967) and in rats (Rabinovitch and Dupré 1974), and in vitro in dogs (Ipp et al. 1977b) and mice (Schatz et al. 1974). On the contrary, some studies could not demonstrate any or only a weak insulin secretory effect of CCK-33 in dogs, rats and man (Rabinovitch and Dupré 1972, 1974, Hedner et al. 1975). The insulin secretion reported in other studies were ascribed to impurities, mainly of GIP, in CCK-preparations used (cf. Brown et al. 1975). However, later studies with the synthetic CCK-8 have shown that this peptide has a clear-cut insulin releasing action in the pig, cat and dog (Frame et al. 1975, Schebalin et al. 1977, Ipp et al. 1977a, Williams and Champagne 1979, Rehfeld et al. 1980). Thus, it seems as if CCK-8 in certain species has an insulin releasing effect in the basal state. Its influence on stimulated insulin secretion is not fully established. Further, the influences of CCK-39 on insulin secretion are not known. This was studied in the present investigation. In addition, the dependency of CCK-8-induced insulin secretion on autonomic receptors, sympathetic nerves, adrenals, amines, and thyroid activity, was studied (papers 1, 7 and 13, and Figs. III and IV).

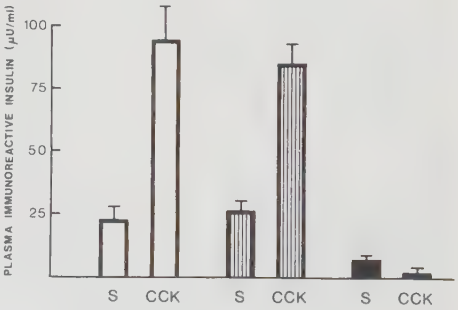
Synthetic CCK-8 and pure porcine CCK-39 were found to be potent insulin secretory agents in the mouse (paper 1). The two polypeptides enhanced, at maximal doses, plasma insulin concentrations by approximately 100 µU/ml. At submaximal doses, CCK-39 was slightly more potent than CCK-8. The peak levels of plasma insulin after a rapid intravenous injection of the two polypeptides were achieved after about 2 minutes; Fig. III shows the plasma insulin concentration at different time intervals after injection of CCK-8. At a dose which was substimulatory on basal insulin secretion, CCK-39 potentiated glucose- and carbachol-stimulated insulin secretion, leaving L-IPNA-stimulated insulin release uninfluenced. At a similar dose, CCK-8 had no effect on stimulated insulin release.

The relation of CCK effects to the muscarinic receptors has been discussed previously. In pancreatic acinar cells no such relation was apparent, since atropine did not block the effects of stimulation of the CCK--receptor (Williams et al. 1978). However, in the guinea pig ileum, CCK-8 has been assumed to exert its effects indirectly, through the release of acetylcholine (Vizi et al. 1973). The results of the present study suggest a relation to both muscarinic and β-adrenergic receptors for the insulin secretory effects of CCK, since methylatropine and L-propranolol depressed the insulin secretory response to CCK-8 and CCK-39 (paper 1).

The influences of chemical sympathectomy, adrenalectomy, the thyroid state, and intracellular accumulation of dopamine on CCK-8-induced insulin release were also studied in this investigation. It was found, that chemical sympathectomy as well as adrenalectomy diminished the insulin secretory effect of CCK-8 (paper 7). Further, the insulin secretory response to CCK-8 was impaired in hypothyroidism and potentiated in hyperthyroidism (paper 13). In addition, after intracellular accumulation of dopamine during an extended period of monoamine oxidase inhibition, the insulin secretory response to CCK-8 was abolished (Fig. IV). The experiments on the influence of intracellular dopamine were performed in analogy with those described in paper 12. It should be noted that basal plasma glucose and insulin concentrations were depressed after the extended period of monoamine oxidase inhibition, which might contribute to the lower insulin secretory response to CCK-8, beside a direct effect of dopamine.

Fig. IV

Plasma concentrations of immunoreactive insulin 2 min after the i.v. injection of saline (S) or CCK-8 (CCK) (6.25 nmol/kg), in mice. The mice were pretreated with saline (open columns) (plasma glucose 7.8 ± 0.2 mmol/l), pargyline + L-DOPA (striped columns) (plasma glucose 7.7 ± 0.4 mmol/l), or 2 doses of pargyline + L-DOPA (black columns) (plasma glucose 2.6 ± 0.4 mmol/l). Mean ± SEM is shown. Each group consisted of 10 animals.



In conclusion, CCK-8 and CCK-39 are potent insulin secretory agents in the basal state in the mouse. Since synthetic CCK-8 had such an effect, which is in agreement with several recent reports in other species (Williams and Champagne 1979, Rehfeld et al. 1980), effects of crude CCK preparations may not entirely be ascribed to impurities in the preparations.

CCK-8 and CCK-39, at similar dose levels, differed in their effects on stimulated insulin secretion. This might illustrate qualitatively different influences, or different metabolism, of CCK-8 and CCK-39.

The effect of CCK-8 on insulin secretion was sensitive to autonomic blockade, to thyroid activity, and to intracellular dopamine accumulation. Further, the sympatho-adrenal system seems to be necessary for the expression of full activity of CCK-8. CCK-8- and carbachol-induced insulin secretion was regulated similarly by certain influences (muscarinic blockade, dopamine accumulation, changes in thyroid activity). Thus, CCK-8 and cholinergic agents seem to have mechanisms in common for their effects on insulin secretion. After β-adrenoceptor blockade, chemical sympathectomy and adrenalectomy, CCK-8- and carbachol-induced insulin secretion were altered differently. These results suggest that CCK-8 does not liberate

acetylcholine or stimulate the cholinergic receptors as its only effect, if at all (cf. Vizi et al. 1973, Williams et al. 1978).

After ß-adrenoceptor blockade or after chemical sympathectomy and adrenalectomy, CCK-8-induced insulin release was impaired. Based on these findings it might be speculated that endogenous ß-adrenoceptor agents sensitize the insulin cells for CCK-8. Thus, CCK-8 seems to be related to both cholinergic and ß-adrenergic agents in its stimulatory effects on insulin secretion.

2. Gastrin

Gastrin was described by Edkins (1905). Its main physiological actions have been found to be stimulation of acid and pepsin secretion, gastric mucosal growth and gastric motility (cf. Walsh and Lam 1980).

Gastrin cells are localized to the antrum and proximal duodenum (McGuigan 1968, Larsson 1979a). These cells contain gastrin-17, but also other peptides seem to occur (Larsson 1979a). Gastrin cells have, furthermore, been suggested to exist in the pancreas (Lomsky et al. 1969, Dockray et al. 1977), but a recent study has indicated that this is true only in the neonatal period (Larsson et al. 1976b). In extracts of the brain, "gastrin--like" material has been found (Vanderhaeghen 1975). Also peripheral nerves, such as nerve fibers in the pancreas, contain immunoreactive gastrin (Larsson 1979b, Rehfeld et al. 1980).

Gastrin has a wide chemical heterogeneity. Gastrins with 34, 17, 14, and 4 amino acids have been described (Rehfeld et al. 1980, Walsh and Lam 1980). The amino acid sequences of the gastrins show a close resemblence to that of the cholecystokinins; in fact, the C-terminal ends of the two polypeptides are identical (Dockray 1977, Mutt 1979). Gastrin is known to be released into the blood after feeding; the main form released seems to be gastrin-17, but gastrin-34 is also liberated (Rehfeld and Stadil 1973, Walsh and Lam 1980).

In earlier studies, crude gastrin preparations (Unger et al. 1967), gastrin-17 (Ipp et al. 1977a, Rehfeld et al. 1978, Lindkaer Jensen et al. 1980), and gastrin-4 (Kikuchi et al. 1971, Rehfeld 1971) have been found to stimulate insulin secretion in man, dogs and pigs. Further, gastrin-17 has, in man, been found to potentiate glucose-stimulated insulin secretion (Dupré et al. 1969), but to be without effect on aminoacid-induced insulin secretion (Rehfeld et al. 1978). Gastrin-17 is reported to potentiate glucose-induced insulin release slightly, when given in a dose comparable to that circulating after feeding (Rehfeld and Stadil 1973). However, if gastrin-17 contributes to the incretin action of the gut, this is probably of minor importance (cf. Budillon et al. 1973). This does not exclude an important effect of gastrin on insulin secretion, if the demonstrated gastrin immunoreactive nerve fibers in pancreas participate in the regulation of hormone secretion from the islets. To further investigate how gastrin-17 influences stimulated insulin secretion, its effects on insulin release stimulated by three different secretagogues were studied *in vivo* in mice. In addition, its effects were related to those of the synthetic gastrin analogue pentagastrin (paper 2).

It was found, that gastrin-17 and pentagastrin slightly elevated plasma insulin concentrations at high doses; at the highest tested dose levels they increased plasma insulin levels by approximately 20 µU/ml. Peak levels of plasma insulin were achieved 2 minutes after the injections. The tetrapeptide gastrin-4 has been found to induce enhancement of plasma IRI of comparable magnitude in man (Rehfeld 1971).

Gastrin-17 and pentagastrin both inhibited L-IPNA-induced insulin secre-

tion, whereas they appeared to have no effect on glucose- or carbachol-stimulated insulin release, at least in doses without direct effects on basal insulin secretion. Since the two polypeptides exerted identical effects on insulin secretion, it is reasonable to assume that they act via the same receptors. It is notable that the effects of gastrin-17 differed from the effects of CCK (papers 1 and 2); the C-terminal four amino acids are identical in these two polypeptides. Thus these four amino acids are not sufficient to yield the large insulin releasing effect observed after administration of CCK-8.

In conclusion, gastrin-17 seems to have a low efficacy on basal insulin secretion; at high doses it elevates basal plasma insulin concentrations by approximately 20 μU/ml. The effect of gastrin-17 and pentagastrin on basal insulin is much weaker than that of CCK-8. Gastrin-17 has no effect on the insulin secretory responses to glucose or to cholinergic stimulation, and has a slight inhibitory influence on the response to β-adrenoceptor stimulation at a dose without effect on basal insulin secretion. These results suggest that gastrin-17 gives only a modest, if any, contribution to the regulation of insulin secretion *in vivo*.

3. Secretin

Secretin has been localized to endocrine cells in the small intestine (Larsson et al. 1977). It is a polypeptide consisting of 27 amino acids; the amino acid sequence shows similarities to that of glucagon, VIP and GIP (Dockray 1977). The main function of secretin is stimulation of water and bicarbonate secretion from pancreas after feeding (cf. Chey et al. 1979).

The effects of secretin on insulin secretion have been the subject of several investigations. Administration of secretin to normal man or dogs has been shown to enhance basal insulin secretion (Unger et al. 1967, Chisholm et al. 1969, Dupré et al. 1969, Dupré et al. 1975, Enk 1976, Lerner 1977, Williams and Champagne 1979, Ebert et al. 1979). Even in diabetics secretin stimulates insulin release (Hindberg et al. 1970, Deckert et al. 1972, Enk 1976). However, when secretin is administered in doses yielding circulating levels comparable to those found physiologically, no effect on basal or glucose-stimulated insulin secretion is seen (Shima et al. 1978, Fahrenkrug et al. 1978). Secretin has been shown to have the capability to potentiate arginine- and glucose-induced insulin secretion, but to be without effect on insulin secretion induced by tolbutamide, glucagon or L-IPNA in man (Dupré et al. 1969, 1975, Lerner 1977). In the dog, secretin potentiates CCK-8-induced insulin secretion (Williams and Champagne 1979). The effects of secretin on basal and stimulated insulin secretion in the mouse were studied in the present investigation (paper 2).

It was found, that secretin at high doses slightly elevated basal plasma insulin levels. After a rapid i.v. injection, peak levels of plasma IRI levels were achieved after 2 min. Secretin exerted only weak effects, weak compared with e.g. glucose, carbachol or CCK. The potency of secretin was equivalent to the potency of gastrin-17. Thus, it is probable that circulating secretin is without an important effect on basal insulin secretion. However, a potential capability of secretin to slightly increase basal insulin levels seems to exist in the mouse.

Secretin potentiated glucose-induced insulin secretion, which is in agreement with earlier studies (Dupré et al. 1969, 1975, Lerner 1977). At the same dose, however, secretin reduced insulin secretion from comparable levels induced by cholinergic stimulation or by β-adrenoceptor stimulation. Specificity for glucose in the potentiating effect on insulin secretion of secretin has been suggested (cf. Lerner 1977). The results

of the present study give credence to that suggestion. Thus, at dose levels without effects on basal insulin secretion, secretin potentiated glucose--induced insulin secretion, and inhibited insulin responses to certain other stimuli.

In conclusion, secretin has the capability to slightly elevate basal insulin secretion, but this effect is seen only at high dose levels. Further, secretin potentiates glucose-induced insulin secretion, and inhibits insulin release after cholinergic and β-adrenoceptor stimulation. In its effects on insulin secretion it does not resemble any other known polypeptide. Secretin is probably of minor importance for insulin secretion under basal conditions, but may participate in interactions with neural, humoral or glucose effects on insulin secretion.

4. Pancreatic Glucagon

In 1921 Banting and Best described a hyperglycemic factor in the pancreas; this factor was later designated glucagon (Banting and Best 1922; Murlin et al. 1923, cf. Unger and Orci 1976). Pancreatic glucagon has been shown to be a single polypeptide chain consisting of 29 amino acids with a molecular weight of 3485 (Bromer et al. 1957). The amino acid sequence of glucagon shows similarities to that of GIP, VIP, and secretin (Dockray 1977).

Pancreatic glucagon has been localized to the A-cells of the pancreatic islets (Baum et al. 1962); also cells in the gastric mucosa of certain mammals contain pancreatic-type glucagon (Larsson et al. 1975). The glucagon cells in the pancreatic islets are in most species located in the periphery of the islets, surrounding the insulin cells (Orci 1976).

Pancreatic glucagon seems to be synthetized from a percursor, proglucagon, which is converted to glucagon after splitting off different fragments (Patzelt et al. 1980, Moody and Sundby 1980). The identity of the various fragments, or glucagon precursors, is yet not known, except in the case of glicentin (see next section).

Glucagon is stored, together with the fragments formed during its synthesis, in the secretory granules of the glucagon cells (Ravazzola et al. 1979). Upon stimulation, the granular content is extruded extracellularly through an exocytotic mechanism (Orci et al. 1970). Thus, glucagon as well as the various fragments formed during glucagon synthesis may influence insulin secretion.

The main effects of glucagon are stimulation of hepatic glycogenolysis, gluconeogenesis and ketogenesis (cf. Cahill 1976). Since glucagon and insulin are produced in adjacent cells, they may regulate each other's release. Glucagon can stimulate insulin secretion, which has been shown *in vivo* in man (Samols et al. 1965, Porte 1967b), dogs (Colwell and Zuckermann 1970) and rats (Turner et al. 1974) and *in vitro* in the rat (Grodsky et al. 1967). Further, insulin has been shown to have the capability to depress glucagon secretion (Östenson 1979), and antiserum against insulin to enhance plasma levels of glucagon (Müller et al. 1971).

Glucagon seems to antagonize certain effects of GIP. This has been demonstrated for lipolysis and cAMP production in fat cells (Dupré et al. 1976) and for glucose-induced insulin secretion in isolated islets (Schäfer and Schatz 1979).

In the present investigation, effects of glucagon on basal, glucose- and cholinergically-induced insulin secretion were tested, and the *in vivo* interactions of GIP and glucagon on insulin secretion were studied. The relation of glucagon-induced insulin secretion to the autonomic receptors was also investigated (papers 3 and 9).

Glucagon induced a moderate, dose-dependent, enhancement of plasma immunoreactive insulin concentrations; peak levels were achieved 2 min after an i.v. injection (Fig. 5 in paper 3, and Fig. III). In maximal doses, glucagon elevated plasma IRI concentrations by approximately 40 μU/ml. An important paracrine effect on insulin cells can thus be suggested for glucagon. Since L-propranolol inhibited glucagon-induced insulin secretion, β-adrenoceptors seem to be necessary for the effect of glucagon. This seems to be in contrast to the muscarinic receptors (paper 9). This is of interest, since cAMP has been suggested to be regulated by β-adrenoceptors and glucagon (Kuo et al. 1973).

Glucagon was, furthermore, found to potentiate glucose-induced insulin secretion (paper 3), and glucagon potentiated the insulin secretory response to cholinergic stimulation. This latter effect of glucagon may be of importance after feeding, when the vagal tone is high, which leads to enhanced glucagon as well as insulin secretion (Kaneto et al. 1974); the latter is then potentiated by glucagon. However, since glucose depresses glucagon secretion (Unger et al. 1962), these intra-islet influences may be of more importance at the beginning of a meal, before any changes in plasma glucose are seen (Steffens 1980). It may be mentioned that glucagon has been shown to also potentiate glibenclamide-induced insulin secretion in the mouse (Ahrén and Lundquist 1980). Thus, glucagon seems to be a general potentiator of stimulated insulin secretion.

To elucidate the eventual interactions between glucagon and GIP on insulin secretion in vivo, a series of experiments was performed (paper 3). Glucagon and GIP appeared to antagonize each other's effect on basal insulin secretion. Contrary, on glucose and carbachol-induced insulin release the two polypeptides showed additive synergism of their effects. The mechanisms behind these effects remain to be established, but it is apparent that different effects of combined stimulation with glucagon and GIP are seen, depending on whether the insulin secretion is stimulated or not.

The gastro-entero-pancreatic localization of glucagon in the mouse and rat was studied by immunocytochemistry (papers 3, 5, and 6). The antiserum which was used in these studies is non-specific with regard to pancreatic glucagon. Hence, cells containing glucagon-like immunoreactivity are also stained (Holst 1978). It was found, that, in the islets, glucagon cells were located in the periphery, surrounding the insulin cells. This mantle distribution was more pronounced in the rat than in the mouse. Glucagon was confined to the secretory granules of the cells. A close association between some glucagon cells and PP cells was noted. In the mouse, glucagon immunoreactivity were also found in scattered endocrine cells in the mucosa of the small and large intestine. It was also demonstrated, that the glucagon cells were stained with some GIP-antisera. The relevance of this is discussed in section 6.

In conclusion, the present investigation has shown that glucagon occurs in peripheral cells in the islets and in endocrine cells scattered in the mucosa of the small and large intestine. Furthermore, glucagon stimulates insulin secretion in vivo; intact β-adrenoceptors seem necessary for this effect. Moreover, glucagon potentiates glucose- and cholinergically-induced insulin release. On basal insulin secretion, glucagon antagonizes the effect of GIP, whereas these two polypeptides show additive synergism in their effects on stimulated insulin secretion.

Glucagon may thus be an important regulator of insulin secretion; it is localized to cells adjacent to insulin cells, stimulates insulin secretion and modulates the insulin secretory response to other secretagogues.

5. Extrapancreatic Glucagon, Glicentin

In 1948 Sutherland and de Duve described a hyperglycemic, glucagon-like factor in the gut, and by radioimmunoassay it was further established that glucagon exists outside the pancreas (Sutherland and de Duve 1948, Samols et al. 1966, Holst 1978). Later it was found, that the glucagon-like immunoreactivity of the gut differed physichochemically and biologically from pancreatic glucagon (Unger et al. 1968). However, glucagon indistinguishable from true pancreatic glucagon was found in the stomach in the dog, but in other species pancreatic glucagon seems to be scarce or absent in the gastrointestinal tract (Larsson et al. 1975, Sundby et al. 1976).

Glucagon-like immunoreactivity in the gut is localized to endocrine cells in the small intestine (Polak et al. 1971). Gut-type glucagon has been demonstrated to be heterogeneous and probably it contains several components (Valverde et al. 1968, Holst 1978). Of all possible glucagon--like components, only one has been highly purified, glicentin (Sundby et al. 1976). Glicentin contains 100 amino acids and has a molecular weight of 11 625 (Jacobsen et al. 1977). Its amino acid sequence has been partially clarified, and evidence has been presented that glicentin contains the full sequence of pancreatic glucagon (Holst 1980). Glicentin has, furthermore, been found in pancreatic glucagon cells, where it occurs in the secretory granules (Ravazzola et al. 1979, Ravazzola and Orci 1980). The exact relation between gut-type glucagon, pancreatic-type glucagon and glicentin is,however,still unsolved (cf. Holst 1978).

The functions of the gut-type glucagon is not known. It may exert hyperglycemic actions or be converted to substances with such actions (Holst 1978), but it may also have functions unrelated to carbohydrate metabolism (cf. Pearse et al. 1977).

The effects of gut-type glucagon on insulin secretion have been studied by several investigators. Since only crude preparations have been available the results are difficult to interpret (Valverde et al. 1968, Gutman et al. 1973, Ohneda et al. 1976, Tanaka et al. 1977). In the present study, effects of highly purified glicentin on insulin secretion were studied (paper 4). Glicentin seems not to be released after feeding (Moody et al. 1978), but may function as a local regulator of insulin secretion, since it probably exists in the secretory granules of the glucagon cells (Ravazzola et al. 1979).

Glicentin was found to partially depress glucose-induced insulin secretion,but to be without influence on basal and carbachol-induced insulin release (paper 4). This indicates that the glucagon cells contain a polypeptide with inhibitory action on glucose-stimulated insulin release. It is of interest,that long-term administration of glicentin to rats has been demonstrated to lead to hyperplasia of the somatostatin cells (Yamada et al. 1980). Whether glicentin stimulates the release of somatostatin is not yet known, but this may be a part of the mechanism whereby glicentin inhibits glucose-induced insulin secretion. However, this cannot be the sole mechanism, since glicentin had no effect on carbachol-induced insulin secretion which was inhibited by somatostatin (Fig. V and paper 5).

6. GIP

GIP is a polypeptide consisting of 43 amino acids (Brown and Dryburgh 1971, cf. Brown et al. 1975), and it shows structural similarities with glucagon, VIP, and secretin (Dockray 1977).

GIP is produced in the small intestine and secreted into the circulation after a meal (Polak et al. 1973, Cataland et al. 1974). It has been shown to stimulate insulin secretion in man (Dupré et al. 1973, Elahi et al.

1979), the dog (Pederson et al. 1975, Ipp et al. 1977a) and the rat (Turner et al. 1974, Taminato et al. 1977, Pederson and Brown 1978, Schäfer and Schatz 1979). This effect has been demonstrated in doses of GIP yielding circulating levels comparable to those normally occurring (Elahi et al. 1979). GIP is glucose-dependent in its effect on insulin secretion, and potentiates glucose- and arginine-induced insulin secretion (Turner et al. 1974, Taminato et al. 1977, Pederson and Brown 1978). It has been suggested that GIP is the main or one of the main incretin candidates (Brown et al. 1975, Creutzfeldt 1979, Elahi et al. 1979).

In the present study, the effects of highly purified GIP on basal, glucose- and carbachol-stimulated insulin secretion were studied *in vivo* in the mouse. In addition, the distribution of GIP immunoreactivity was studied by immunocytochemistry (paper 3).

GIP was found to stimulate basal insulin secretion in a dose-dependent manner; peak levels were achieved 2 minutes after the injection (Fig. III). GIP increased plasma IRI, at maximal dose levels, by approximately 40 µU/ml. GIP was thus equipotent to glucagon, but weaker than CCK-8 and CCK-39 in this respect. Earlier studies have demonstrated a threshold level (5.5 mmol/l) for the surrounding glucose concentrations, under which GIP has no effect on insulin secretion (Brown et al. 1975). In the non-fasted mouse, plasma glucose (approximately 8 mmol/l) is above this level.

GIP potentiated glucose and carbachol-induced insulin secretion. Thus, the effects of GIP showed strong resemblance to those of glucagon. These two polypeptides antagonized each other's effect on basal insulin secretion, whereas they showed additive synergism in their effects on stimulated insulin secretion. An interaction between the effects of GIP and glucagon has earlier been demonstrated *in vitro* in fat cells and in isolated islets (Dupré et al. 1976, Schäfer and Schatz 1979). Glicentin was without apparent influences on the effects of GIP (papers 3 and 4).

Thus, GIP was found to exert stimulatory effects on the insulin cells, both with regard to basal and to stimulated insulin secretion. In case GIP is released after feeding in the mouse, it may be an important incretin factor also in this species.

GIP immunoreactivity has earlier been found in the glucagon cells in the rat, guinea pig, cat, dog, pig and man (Smith et al. 1977, Alumets et al. 1978). In the present study, four different antisera against GIP were used in order to throw additional light on the occurrence of GIP in the glucagon cells (paper 3). Three of these GIP-antisera gave a strong reaction with material in the glucagon cells in the islets and in the gut; the material was localized to the secretory granules. Since not all GIP antisera reacted with the glucagon cells, the immunogenic peptide is not natural GIP. It may instead represent some amino acid sequences in secretory granule material which not immunologically react with the fourth antiserum. However, the GIP-like material may be chemically related to GIP, since it reacts with several GIP-antisera. Since it is localized to secretory granules, it may be released concomitantly with glucagon. If the GIP-like peptide possesses GIP-like biologigal activity, the demonstrated effects of GIP on insulin secretion, and the interactions with the effects of glucagon, might represent paracrine regulatory mechanisms. The glucagon cells might thus contain substances with positive (glucagon, the GIP-like peptide) as well as negative (glicentin) effects on glucose-induced insulin secretion in the mouse.

7. VIP

VIP was originally isolated from porcine small intestine (Said and Mutt 1970) and found to be a 28 amino acid polypeptide with structural simi-

larities to secretin, glucagon, and GIP (Mutt and Said 1974, Dockray 1977). VIP has been found to have a wide spectrum of biological activities. It induces vasodilatation, stimulation of glycogenolysis and lipolysis, secretion from small intestine and exocrine pancreas, and inhibition of gastric acid secretion, beside its effects on hormonal release from the endocrine pancreas (cf. Fahrenkrug 1979).

Originally, VIP was assumed to be localized to endocrine cells in the gut (Polak et al. 1974), but later studies revealed VIP immunoreactivity in nerve fibers only (Larsson et al. 1976c, cf. Fahrenkrug 1979). VIP nerves seem to have a ubiquitous distribution; they are found in the central nervous system, and in the digestive, genitourinary and upper respiratory tracts, in the pancreas and the thyroid, in the vagus, and in striated muscles (Larsson et al. 1976c, Sundler et al. 1978, Fahrenkrug 1979, Larsson and Rehfeld 1979, Bishop et al. 1980, Järhult et al. 1980, Ahrén et al. 1980).

In several species, VIP nerves have been demonstrated in the pancreas. The nerves are seen in the exocrine parenchyma, and they are seen in intimate association with small blood vessels. VIP nerve fibers have also been found closely to the islets as a peri-insular network and penetrating the islets (Sundler et al. 1978, Bishop et al. 1980). Pancreatic ganglia also contain VIP fibers (Larsson and Rehfeld 1979). VIP has not been localized to endocrine islet cells (Sundler et al. 1978).

Thus, the morphological basis for a neurocrine influence, rather than an endocrine/paracrine, by VIP on pancreatic functions exists. VIP has been found to have potential capability to stimulate insulin secretion. This has been demonstrated in the perfused porcine (Lindkaer Jensen et al. 1978), dog (Hermansen 1980) and rat pancreas (Szecówka et al. 1980), and *in vivo*, dogs (Kaneto et al. 1977, Ohneda et al. 1977). *In vivo* in the rat, no effect was seen (Turner et al. 1974). VIP has been shown to potentiate glucose-induced insulin secretion, but to be without effect on arginine-induced (Szecówka et al. 1980). The effects of VIP on stimulated insulin secretion *in vivo* are not established and were, together with effects on basal insulin levels, studied in this investigation (paper 2).

It was found, that VIP induced a very slight elevation of basal plasma insulin levels. This confirms the study of Turner et al. (1974) and is in contrast to *in vitro* experiments, in which VIP has been found to have strong influences on insulin secretion (see above). Perhaps the local concentration of VIP in the islets after an i.v. injection is too low to exert strong effects. Alternatively, indirect vasodilatatory effects of VIP might counteract a direct stimulatory effect on insulin secretion.

VIP was in the present study found to enhance glucose- and L-IPNA-induced insulin secretion, but to be without effect, at the same dose, on carbachol-induced insulin release. Earlier, VIP has been shown to potentiate glucose-stimulated insulin secretion *in vitro* (Szecówka et al. 1980). VIP stimulates the production of cAMP in several organs (Fahrenkrug et al. 1979, Ahrén et al. 1980), and it is of interest that VIP enhanced the insulin secretory responses to glucose and L-IPNA, both which are more or less dependent on cAMP (Kuo et al. 1973, Hedeskov 1980).

In conclusion, VIP exerts only a very weak effect on basal insulin secretion *in vivo*, whereas it potentiates the insulin secretory responses to glucose and β-adrenoceptor stimulation. The results do not refute an assumption that VIP is a local regulator of stimulated insulin secretion.

8. Somatostatin

Somatostatin is a tetradecapeptide originally isolated from hypothalamus

and initially found to inhibit growth hormone secretion (Brazeau et al. 1973). Later, somatostatin was found in other parts of the brain, in peripheral nerves and in endocrine cells in the pancreatic islets, gut and thyroid (Luft et al. 1974, Dubois 1975, Hökfelt et al. 1975a, cf. Luft et al. 1978).

Somatostatin has been shown to exert a variety of effects. Beside its initially described inhibitory influence on growth hormone secretion, it also inhibits the secretion of TSH and a variety of gastrointestinal polypeptides, such as gastrin, secretin, CCK, gut-type glucagon, VIP, and GIP (cf. Raptis et al. 1978, Luft et al. 1978). It also inhibits thyroid hormone secretion (Ahrén et al. 1978), gastric acid secretion, and motility in the gastrointestinal tract (Luft et al. 1978). Further, it inhibits splanchnic blood flow and carbohydrate absorption (Wahren and Felig 1976). In addition, somatostatin exerts powerful inhibitory effects on the secretion of insulin and glucagon from pancreatic islets (Alberti et al. 1973, Mortimer et al. 1974, Koerker et al. 1974, Efendić and Luft 1975).

Because of all these effects, its patchy distribution in the body, and its short half life in blood, it has been proposed that somatostatin is acting in a paracrine manner at the site of its production (Pearse et al. 1977, Alumets et al. 1979, Larsson et al. 1979).

The mechanisms of its actions and the possible importance of somatostatin in the intra-islet regulation of hormone release have been studied earlier. The insulin secretory responses to several stimuli have been shown to be inhibited by somatostatin in a variety of experimental conditions. Thus, insulin secretion stimulated in vivo by glucose, IPNA, arginine, glucagon, tolbutamide, and secretin has been shown to be inhibited by somatostatin (Koerker et al. 1974, Chideckel et al. 1975, Efendić et al. 1976, 1978b, Taborsky et al. 1979). Somatostatin has also been found to depress insulin secretion in vitro (Alberti et al. 1973, Claro et al. 1977, Moltz et al. 1977, Grodsky et al. 1977) which indicates that it has a direct effect on the insulin cells.

Since somatostatin inhibits the effects of a variety of stimuli, it has been suggested that it interferes with an important step in the insulin discharge process. Evidences have been published suggesting an effect of somatostatin on Ca^{++}-uptake (Bhathena et al. 1976, Oliver 1976) or the intracellular translocation of Ca^{++} (Grodsky et al. 1977). Since somatostatin exerts inhibitory effects on insulin secretion stimulated by exogenous cAMP and inhibitors of phosphodiesterase (Gerich et al. 1975, Iversen and Hermansen 1980), effect of somatostatin on cAMP metabolism (cf. Claro et al. 1977) is probably of minor importance for its effects on hormone secretion.

Another possibility is that somatostatin activates the α-adrenoceptors, since stimulation of these receptors inhibits insulin secretion (cf. Woods and Porte 1974). It was shown in vivo in dogs that the α-adrenoceptor blocker phentolamine prevented the inhibitory effect of somatostatin on insulin secretion (Smith et al. 1976); a similar result was obtained in vitro (Basabe et al. 1977). However, several reports from various species have ruled out that somatostatin acts by stimulation of α-adrenoceptors (Efendić and Luft 1975, Pace et al. 1977, Kaneto et al. 1978a, Efendić et al. 1978b, Järhult et al. 1979, Iversen and Hermansen 1980).

The aim of this study was to investigate if certain insulin secretagogues are less sensitive to somatostatin in their effects on insulin secretion. Of special interest was the sensitivity to somatostatin after adrenergic stimulation. Taborsky and associates have indicated that IPNA-induced insulin release seems less sensitive to somatostatin than glucose-induced (Taborsky et al. 1979). Further, splanchnic nerve stimulation

or indirect sympatho-adrenal discharge enhance glucagon secretion through a mechanism which seems insensitive to somatostatin (Järhult et al. 1978, Bloom and Edwards 1978). Beside the effects on insulin secretion, cellular distribution in the pancreas and gut of somatostatin was studied in the present investigation (papers 5, 6, 10, 11, and 12, Figs. V and VI).

Somatostatin was localized to cells in the islet periphery in the mouse and rat (papers 5 and 6). Furthermore, these cells differed from other islet cells in that they did not take up and decarboxylate L-DOPA or L-5--HTP (paper 12).

In the mouse, somatostatin was shown to inhibit the insulin secretory response to glucose, carbachol, and L-IPNA *in vivo* (paper 5, Figs. V and VI). The action of somatostatin on L-IPNA-induced insulin secretion was less intense than on glucose- or carbachol-induced insulin secretion. This indicates that β-adrenoceptor stimulation is less sensitivity to somatostatin than glucose or cholinergic stimulation in the mouse. Furthermore, somatostatin more potently inhibited insulin secretion after carbachol than after glucose.

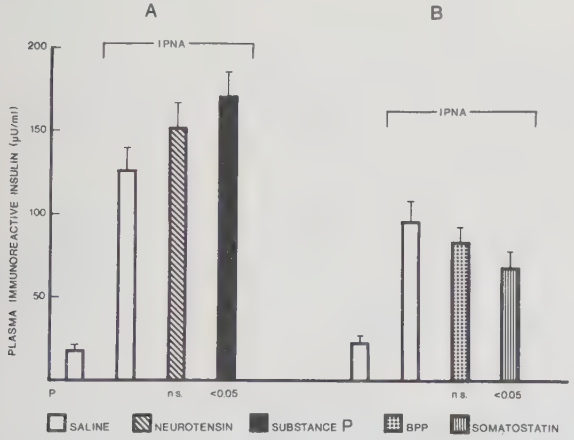


Fig. V

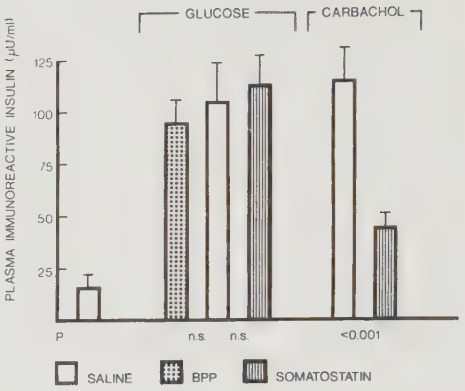
Plasma concentrations of immunoreactive insulin 5.5 min after the i.v. injection of L-IPNA (0.34 μmol/kg). Neurotensin, substance P, BPP, or somatostatin (4.25 nmol/kg), was injected 15 sec prior to L-IPNA in mice. Controls were injected with saline. Mean ± SEM is shown. Each group consisted of 12-16 animals. P indicates probability level of random difference *vs* the group injected with saline + L-IPNA. n.s. indicates not significant. Two different series of experiments were performed (Fig. V A and B).

In the rat, somatostatin infusion, 0.1 μg/min, depressed basal insulin secretion and inhibited glucose-stimulated insulin release (papers 10 and 11). Moreover, somatostatin inhibited insulin secretion after α-adrenoceptor blockade and β-adrenoceptor stimulation. β-Adrenoceptor stimulated insulin release was less affected by somatostatin than glucose-stimulated. Thus, both in the mouse and rat, a partial insensitivity for somatostatin was seen after β-adrenoceptor stimulation. This agrees with the results by Taborsky et al. (1979) in the dog, though in their study insulin levels after glucose and β-adrenoceptor stimulation were not comparable. A decreased sensitivity to somatostatin was also noted in the rat after surgical splanchnicotomy and adrenalectomy, but only if the α-adrenoceptors were blocked and the β-adrenoceptors were intact (paper 10).

A β-adrenoceptor stimulation thus seems to induce mechanisms which are less sensitive to somatostatin than e.g. glucose effects. An α-adrenoceptor blockade, however, which shows similarities to a β-adrenoceptor stimulation, does not induce a decreased sensitivity to somatostatin; the splanchnic nerves must be cut to see it. Thus, the splanchnic nerves contribute to the sensitivity to somatostatin in the insulin secretory machinery. This effect of the splanchnic nerves may be exerted by a substance with α-adrenoceptor stimulatory properties, or by a peptide confined to the nerves (cf.

Fig. VI

Plasma concentrations of immunoreactive insulin 2 min after the i.v. injection of D-glucose (2.78 mmol/kg) or carbachol (0.16 µmol/kg) in mice. BPP or somatostatin (0.85 nmol/kg) was injected 15 sec prior to D-glucose or carbachol. Controls were injected with saline. Mean ± SEM is shown. Each group consisted of 12-16 animals. P indicates probability level of random difference between groups. n.s. indicates not significant.



Lundberg et al. 1978). It is notable that after 6-hydroxydopamine treatment, when the adrenergic terminals are destroyed, somatostatin has insulin lowering effect during α-adrenoceptor blockade, which is contrary to after surgical splanchnicotomy. The sensitivity to somatostatin seems retained after β-adrenoceptor blockade in splanchnicotomized-adrenalectomized rats during α-adrenoceptor blockade. Thus, enhanced endogenous β-adrenoceptor effects contribute to the decreased potency of somatostatin after splanchnicotomy, adrenalectomy and α-adrenoceptor blockade.

In conclusion, somatostatin inhibits basal insulin secretion and the insulin secretory responses to glucose, to cholinergic and to β-adrenoceptor stimulation. The effects of somatostatin are most potent after stimulation with glucose and the cholinergic agonist carbachol. Thus, somatostatin seems to exert influences on processes linked more to cholinergic or glucose stimulation than to β-adrenoceptor stimulation.

9. PP

PP was originally isolated from chicken pancreas (Kimmel et al. 1968) but found later to occur also in mammals (Lin et al. 1973). It has been found to be a polypeptide consisting of 36 amino acids(Kimmel et al. 1975); bovine PP (BPP) and avian PP (APP) have in common 18 of these.

PP is localized to a discrete cell population in the islets, and in some species, PP cells are also found scattered over the exocrine pancreas; in the mouse the main location is in the islets (Larsson et al. 1976a). PP cells are more numerous in the duodenal part of the pancreas than in the splenic (Larsson et al. 1976a). Some species, e.g. the opossum, have PP cells also in the gut (Larsson et al. 1976a). In addition, PP-like material has been found in central and peripheral nerves, e.g. in the gut (Lorén et al. 1979).

The physiological functions of PP are not known. Since PP seems to occur in secretory granules (Larsson et al. 1976a) it may be secreted, and thus function as a regulatory substance in a paracrine or endocrine action. APP has been shown to stimulate hepatic glycogenolysis in chickens, but no effect has been found on blood glucose (Hazelwood et al. 1973). Further, BPP inhibits pancreatic enzyme secretion, and stimulates gastric acid secretion in the dog (Lin et al. 1973).

In the present study, the effects on insulin secretion and the cellular

localization of BPP in the mouse were investigated (papers 5 and 6). PP cells were demonstrated in the periphery of the islets; they were more numerous in the duodenal part of the pancreas. A close association between PP cells and some glucagon cells was noted.

BPP inhibited glucose-induced insulin release, but was without effect on insulin secretion induced by carbachol and L-IPNA (paper 5, and Figs. V and VI). The capability of BPP to reduce the glucose effect was weaker than that of somatostatin and glicentin (cf. paper 5, paper 4, and Fig. VI). BPP was without effects on basal insulin secretion. Recently, PP was reported to inhibit secretin-induced insulin secretion in vivo in dogs, but to be without effect on basal or CCK-8-induced insulin secretion (William and Champagne 1979).

From these studies it is difficult to establish a role for PP in regulating insulin secretion. However, the potential capability of PP to regulate insulin secretion in a paracrine manner exists. The inhibitory action of PP on glucose-induced insulin secretion might be of importance in diabetes, where high plasma PP levels have been observed (cf. Floyd et al. 1980).

10. Substance P

Substance P is an 11 amino acid polypeptide which initially was discovered in the gut and brain (v Euler and Gaddum 1931, Mroz and Leeman 1977). Substance P has been localized to endocrine cells in the gastrointestinal tract (Pearse and Polak 1975) and to central and peripheral nerves (Hökfelt et al. 1975b, cf. Snyder and Innis 1979). Substance P-containing nerves have also been found in the pancreas (Larsson and Rehfeld 1979).

Substance P has been found to induce hypotension, gut contraction and hyperglycemia (Brown and Vale 1976, Mroz and Leeman 1977, Kaneto et al. 1978).

Substance P has, furthermore, been shown to stimulate glucagon and somatostatin secretion (Brown and Vale 1976, Moltz et al. 1977, Kaneto et al. 1978b, Hermansen 1980). Its role in the regulation of insulin secretion is, however, not established. Injection of substance P into rats (Brown and Vale 1976) and dogs (Sasaki 1976) depressed insulin secretion, but infusion into dogs enhanced it (Kaneto et al. 1978b). In the perfused dog pancreas, substance P potentiated glucose-induced insulin secretion (Hermansen 1980), and in the perfused rat pancreas, it inhibited arginine-induced insulin release (Efendić et al. 1977). In isolated rat islets, substance P inhibited glucose-induced insulin secretion (Moltz et al. 1977). In in vivo experiments the effects of substance P on blood pressure and blood glucose might mask direct effects on insulin secretion, but the diverse effects found in in vitro studies are more difficult to explain.

In the present study (paper 5 and Fig. V), the gastro-entero-pancreatic cellular distribution of substance P and effects on insulin secretion were investigated in the mouse. Substance P was localized to endocrine cells and nerves in the gut. Substance P was found to be without effect on basal insulin and glucose concentrations at the dose levels studied. At the dose of 4.25 nmol/kg, substance P inhibited the insulin-secretory response to glucose, was without effect on the insulin response to carbachol and stimulated the insulin response to L-IPNA (paper 5 and Fig. V). Moreover, after increasing the dose of substance P ten-fold, it was found that the polypeptide was without effect on glucose-induced insulin secretion. These effects are probably not due to changes in blood pressure, since the effects of substance P were different depending on the nature of the secretagogues, and since the inhibitory effect on glucose-induced insulin release was prevented by increasing the dose. The varying effects depending on the

nature of the stimuli and on the dosage may explain the varying results obtained in the different published studies.

11. Neurotensin

Neurotensin is a 13 amino acid polypeptide, originally found in the hypothalamus (Carraway and Leeman 1973, 1975). However, most of the neurotensin in the body is localized to discrete endocrine cells in the distal part of the small intestine (Orci et al. 1976, Helmstaedter et al. 1977, Sundler et al. 1977a, 1977b).

Neurotensin has been demonstrated to induce hypotension and hepatic glycogenolysis, causing hyperglycemia (Carraway and Leeman 1973). The results of studies of the effects of neurotensin on hormone release from endocrine pancreas are controversial. Most *in vivo* studies have demonstrated a stimulated insulin secretion after neurotensin administration; this has been shown in anaesthetized dogs (Ukai et al. 1977, Kaneto et al. 1978b, Ishida 1977) and conscious calves (Blackburn et al. 1980). However, inhibited or uninfluenced insulin secretion was demonstrated in anaesthetized rats (Brown and Vale 1976, Nagai and Frohman 1978).

In most studies, changes in plasma insulin concentrations preceded those in plasma glucose, and neurotensin was found to stimulate insulin secretion without affecting blood pressure (Blackburn et al. 1980). This indicates that neurotensin might directly influence insulin secretion, and this is further corroborated by *in vitro* studies.

From *in vitro* studies (rat islets) the influences of neurotensin on insulin secretion were shown to be highly dependent on the glucose concentrations in the surrounding medium. At high glucose concentration, the polypeptide inhibited, and at low glucose concentration, it stimulated insulin secretion. Neurotensin was also found to inhibit arginine-induced insulin secretion, but to be without effect on the insulin secretory response to glucagon (Moltz et al. 1977, Dolais-Kitabgi et al. 1979, Dolais-Kitabgi and Freychet 1979).

In the present investigation, the cellular distribution and effects on insulin secretion of neurotensin were studied in the mouse (paper 5 and Fig. V). Neurotensin was found to occur in the gut; most neurotensin cells were localized to the epithelium of jejunum and ileum and a few in the colon. No neurotensin cells were detected in the pancreas.

Neurotensin had no influence on basal insulin secretion and no effect was exerted on the insulin secretory responses to glucose or L-IPNA (paper 5 and Fig. V). However, neurotensin potentiated carbachol-induced insulin release. It seems unlikely that neurotensin-induced hypotension would solely explain this effect, since a hypotension-induced sympatho-adrenal discharge inhibits insulin secretion (Järhult and Holst 1978). Also, neurotensin did not change plasma glucose during 2 minutes after its injection. Thus, it is reasonable to assume that the effects of cholinergic stimulation on insulin secretion is directly modulated by neurotensin, and that cholinergically induced insulin secretion is more sensitive to neurotensin than the insulin response to other secretagogues. It is of interest that L-IPNA-induced insulin secretion was uninfluenced by neurotensin, since it was earlier suggested that neurotensin influenced the islets by a mechanism independent of adenylate cyclase-cyclic AMP-system (Dolais-Kitabgi and Freychet 1979).

Whether neurotensin might have the capability to influence the islets physiologically is not known. Neurotensin has not been found in the pancreas, and it has a short half-life in the circulation (1.4 min) (Blackburn et al. 1980). However, the polypeptide exists in the gut and is released

into the blood after feeding (Blackburn and Bloom 1979), and may then e.g. potentiate cholinergically induced insulin secretion.

12. Summary

The main purpose of the present investigation (papers 1-13) was to characterize the possible modulation of insulin secretion exerted by various gastro-entero-pancreatic polypeptides in the conscious mouse. A total of 13 polypeptides were used, and table III summarizes the effects on basal plasma insulin concentrations and on the insulin response to various stimuli exerted by these polypeptides. It has earlier been shown, that changes in pancreatic blood flow are not of critical importance for alterations in insulin secretion (Rappaport et al. 1971). Further, changes in plasma insulin levels after polypeptide administration were always observed without any alterations in plasma glucose concentrations. Thus, the observed effects of the polypeptides are assumed to depend mainly on direct effects on insulin secretion, and not on changes in blood flow, alterations in peripheral uptake of insulin, or primary effects on plasma glucose.

Table III

Summary of the Effects on Insulin Secretion of the Polypeptides in the Mouse

Peptide	Basal Insulin	Insulin secretion stimulated by glucose	carbachol	L-IPNA
CCK-39	+++	+	+	0
CCK-8	+++	0	0	0
Gastrin-17	+	0	0	-
Pentagastrin	+	0	0	-
Secretin	+	+	-	-
Glucagon	++	+	+	nt
Glicentin	0	-	0	nt
GIP	++	+	+	nt
VIP	+	+	0	+
Somatostatin	-*	-	--	-
BPP	0	-	0	0
Substance P	0	(-)	0	+
Neurotensin	0	0	+	0

+ indicates a different degree of stimulation, - indicates a different degree of inhibition, 0 indicates no effect and nt indicates not tested.

* indicates in the rat

It may be noted, that the polypeptides used were either the porcine, bovine or human form; it cannot be excluded that murine polypeptides might have had somewhat different effects. In any case, CCK-39 and CCK-8 were the most efficient polypeptides on basal insulin secretion. Glucagon and GIP had moderate effects, whereas the other peptides had no or slight influences on basal insulin during the time-period studied.

The effects on stimulated insulin secretion varied between the polypeptides. This indicates that they have different mechanisms of action. Further, the effects of the polypeptides on stimulated insulin secretion were highly dependent on the nature of the secretagogue. This, in turn, indicates that separate or partially separate secretory pathways may exist for glucose, carbachol, and L-IPNA, respectively. Moreover, the results show that caution is necessary when interpreting results of polypeptides on stimulated insulin secretion. It may be speculated, that the various polypeptides are important in certain occasions, e.g. neurotensin when the cholinergic tone is high, and GIP and somatostatin when plasma glucose concentrations are elevated.

IV. INFLUENCES OF THE AUTONOMIC NERVOUS SYSTEM ON INSULIN SECRETION

A. General Considerations

The pancreatic islets have a rich supply of autonomic nerves (cf. Langerhans 1869, v Campenhout 1927, Woods and Porte 1974, Gerich and Lorenzi 1978, Edwards and Bloom 1978, Jacoby and Bryce 1978). These nerves consist of cholinergic, adrenergic and peptidergic fibers. They enter the islets accompanying blood vessels (Coupland 1958, Cegrell 1968, Lundquist and Ericson 1978, Bishop et al. 1980). Gap junctions have been demonstrated between nerve endings and islet endocrine cells, which shows that a close connection between neurons and endocrine cells exists (Orci et al. 1973). Nerve terminals are also found at some distance from endocrine cells, and a single nerve terminal may thus influence several cells via release of its neurotransmitter (Kobayashi and Fujita 1969). The influence of the various nerves on insulin secretion has been the subject of several studies (Woods and Porte 1974).

1. The Cholinergic Nervous System and Insulin Secretion

Electrical stimulation of the vagus enhances insulin release in the dog and the rabbit (Frohman et al. 1967, Findlay et al. 1969, Bergman and Miller 1973, Marliss et al. 1973, Porte et al. 1973, Kaneto et al. 1974). The same effect has acetylcholine (Iversen 1973b, Kaneto and Kosaka 1974, Burr et al. 1976). Cholinergically induced insulin secretion is inhibited by atropine (Frohman et al. 1967, Findlay et al. 1969, Kaneto and Kosaka 1974). Vagotomy seems to decrease insulin secretion in the dog (Frohman et al. 1967). Several authors report no effect on, or a slight decrease of basal insulin secretion by atropine (Porte et al. 1973, Bloom et al. 1974a, 1974b, 1978). Atropine has been shown to be without effect on glucose-induced insulin secretion in man (Henderson et al. 1976). In conscious calves, however, it was recently reported that atropine markedly attenuated the insulin release in response to glucose (Bloom and Edwards 1980). It has been suggested that the early insulin response to a meal is mediated by the vagus nerves, and that the physiological role of the vagus on insulin secretion is in relation to feeding (Edwards and Bloom 1978, Steffens 1980).

2. The Adrenergic Nervous System and Insulin Secretion

The islets are innervated by the adrenergic splanchnic nerves (cf. Woods and Porte 1974). Electrical stimulation of these nerves inhibits insulin secretion in dogs and calves (Porte et al. 1973, Bloom and Edwards 1975), and reflex activation of the sympatho-adrenal system depresses basal insulin secretion in the cat (Järhult and Holst 1978).

α-Adrenoceptor stimulation by noradrenaline and adrenaline depresses insulin secretion *in vivo* in man (Porte and Williams 1966, Porte 1967a, Robertson and Porte 1973, Efendić et al. 1978a). Conversely, β-adrenoceptor stimulation enhances insulin secretion (Porte 1967b, Loubatières et al. 1971). Since β-adrenoceptor blockade by propranolol has been demonstrated to depress basal plasma insulin levels (Gagliardino et al. 1970, Robertson and Porte 1973), intact β-adrenoceptors seem to be a prerequisite for normal basal insulin secretion *in vivo*. However, a lack of effect of propranolol in this respect has also been reported (Cerasi et al. 1972). Blockade of α-adrenoceptors by phentolamine leads to an elevated concentration of plasma insulin (Gagliardino et al. 1970, Robertson and Porte 1973). Thus, it has been suggested that basal insulin secretion *in vivo* is modulated by a functional balance between α- and β-adrenoceptor activity (Lundquist 1971, Robertson and Porte 1973, Goodner et al. 1973).

Glucose-induced insulin release has been reported to be depressed by β-adrenoceptor blockade (Cerasi et al. 1972) and α-adrenoceptor stimulation (Porte 1967a, Efendić et al. 1978a). The importance of the adrenoceptors for stimuli to insulin secretion other than glucose is not yet clearly established. Recently, it was reported that adrenaline more potently inhibited the insulin secretory response to glucose than the response to glucagon, arginine or tolbutamide in man (Efendić et al. 1978a). Similarly, in the dog, adrenaline more potently inhibited insulin secretion after glucose stimulation than after glucagon stimulation (Colwell and Zuckerman 1970).

It has recently been demonstrated that the splanchnic nerves are stained with antisera against several polypeptides, such as somatostatin, substance P, VIP, CCK, and met-enkephalin (Lundberg et al. 1978). In addition, VIP- and CCK-containing nerves seem to innervate pancreatic islets (Bishop et al. 1980, Rehfeld et al. 1980). The exact role of these nerve fibers in the regulation of pancreatic hormone release has not yet been established.

In conclusion, it seems established that the vagus nerve can enhance and the splanchnic nerves depress insulin secretion. Also, stimulation of α-adrenoceptors inhibit and of β-adrenoceptors stimulate insulin secretion. The relative importance of α- and β-adrenoceptors in the regulation of insulin secretion has been shown to vary between species and, further, to depend on the age of the animal. Thus, insulin secretion in the dog seems to be more sensitive to β-adrenoceptor stimulation than insulin secretion in the rat, and, in the adult dog, β-adrenoceptor stimulation evokes a more marked insulin secretion than in the newborn dog (Loubatières-Mariani et al. 1977).

The effects of the autonomic nervous system on stimulated insulin secretion is less well characterized, as is the role of the autonomic nerves in regulation of basal insulin secretion. These subjects were studied in this investigation. The influences of autonomic blockade by propranolol and methylatropine, of chemical sympathectomy and of adrenalectomy on basal and stimulated insulin secretion were studied in the conscious mouse. In addition, the influences of chemical sympathectomy, splanchnic denervation and β-adrenoceptor stimulation on insulin secretion were investigated in the rat (papers 1, 6, 7, 8, 9, 10,and 11).

B. Present Results

1. Sympatho-Adrenal System and Basal Insulin Secretion

In the first series of experiments, the adrenergic innervation and the influence of chemical and surgical sympathectomy, adrenalectomy and β-adrenoceptor blockade on basal insulin secretion were studied in the mouse and rat (papers 1, 6, 7, 9,and 10). By fluorescence histochemistry, numerous adrenergic nerve fibers were demonstrated in pancreatic islets of the normal mouse and rat. They entered the islets in close connection with small blood vessels, and were mostly located in the periphery of the islets. The mouse seemed to harbour more islet adrenergic nerves than the rat. 6-Hydroxydopamine, which selectively destroys adrenergic nerve terminals (Kostrzewa and Jacobowitz 1974), induced a substantial reduction in the number of islet adrenergic nerves. The remaining nerves appeared damaged. Thus, a chemical sympathectomy was induced. The drug did not induce any detectable damage to, or changes in, the islet endocrine cells (paper 6).

In the conscious mouse, basal plasma insulin levels were decreased after

6-hydroxydopamine treatment as well as after adrenalectomy and β-adrenoceptor blockade (papers 1, 6, 7,and 9). Hence, adrenergic nerves seem to exert a promoting influence on basal insulin secretion in the mouse. In addition, the adrenal hormones seem to be of importance for maintaining basal insulin levels.

In the rat, on the other hand, chemical sympathectomy by 6-hydroxydopamine administration enhanced basal insulin secretion (papers 6 and 10). This is in agreement with an earlier report (Burr et al. 1974). Species differences thus seem to exist with regard to the influence of the sympatho-adrenal system on basal insulin secretion. In the adrenalectomized rat, surgical splanchnicotomy induced a pronounced enhancement of insulin secretion, much more pronounced than was seen after chemical sympathectomy (paper 10). This elevation of insulin secretion could be inhibited to normal values by propranolol. Thus, surgical splanchnicotomy seems to induce functional alterations in the balance between α- and β-adrenoceptor influences, which are more pronounced than after chemical sympathectomy. Fibers unaffected by 6-hydroxydopamine, but affected by the surgical procedure, might be of importance for the differences between surgical and chemical sympathectomy. Surgical splanchnicotomy affect also non-adrenergic neurons, notably peptidergic (cf. Lundberg et al. 1978), whereas after 6-hydroxydopamine treatment only the adrenergic nerve fibers are assumed to be affected. The peptidergic fibers, together with the functional balance between α- and β-adrenoceptors, thus may be of importance for the influences on insulin secretion by the splanchnic nerves.

In conclusion, the splanchnic nerves exert, in the rat, a strong inhibitory tonus on insulin secretion. It is of interest to note that plasma glucose levels appeared to be largely unaffected by surgical splanchnicotomy, despite the observed changes in plasma insulin concentrations. In the unanaesthetized mouse, the sympatho-adrenal system promotes basal insulin secretion.

2. Sympatho-Adrenal System and Stimulated Insulin Secretion

The effects of chemical sympathectomy and/or adrenalectomy on stimulated insulin secretion were investigated in a series of experiments in the conscious mouse (paper 7). It was found,that after chemical sympathectomy by 6-hydroxydopamine treatment, insulin secretion induced by terbutaline, carbachol or glucose was enhanced and by CCK-8 inhibited. After adrenalectomy, the insulin secretory response to CCK-8 was impaired,and to glucose and carbachol increased. The time after manipulation with the sympatho--adrenal system was of importance for the expression of some of the effects. Thus, the influences on glucose-induced insulin secretion took time to develop, whereas the influences on CCK-8-induced insulin secretion vanished with time. These influences of the sympatho-adrenal system on stimulated insulin secretion might be explained in several ways.

The secretagogues might in the normal animals influence the neural or adrenomedullary release of catecholamines (cf. Westfall 1977). After elimination of the sympatho-adrenal system, such influences are prevented. Manipulations with the sympatho-adrenal system might in addition be followed by sensitivity changes in receptors, with a subsequent alteration in insulin response to stimulation (Haeusler et al. 1969). Receptor supersensitivity might cause the observed potentiated insulin secretory response to terbutaline after chemical sympathectomy. The effects after chemical sympathectomy and adrenalectomy might, additionally, be explained by elimination of the counterregulatory tonus exerted by catecholamines (cf. Efendić et al. 1978a), and the effects after adrenalectomy by the eliminated

influences of glucocorticoids Plasma glucose was lowered after the combined treatment of chemical sympathectomy and adrenalectomy, which might influence subsequent insulin response to stimulation. Both glucose- and carbachol-induced insulin secretion were, however, enhanced after chemical sympathectomy, despite the lowered plasma glucose. Finally, direct effects of 6-hydroxydopamine on islet cell function might affect the response to stimulation .

In conclusion, 6-hydroxydopamine and adrenalectomy induced alterations in the magnitude of insulin secretion after stimulation with terbutaline, glucose, CCK-8 and carbachol. The results might be explained by a variety of events induced by the partial chemical sympathectomy and adrenalectomy. They indicate, however, that the adrenergic nerves, endogenous adrenal hormones, and adrenoceptors are of importance for insulin secretion stimulated by these chemically different secretagogues.

Earlier, a reduced glucose-induced insulin secretion was reported after long-term chemical sympathectomy in the rat (Burr et al. 1974). This was confirmed in the present study (paper 7). On the other hand, glucose-induced insulin release in the mouse was enhanced after long-term chemical sympathectomy. Thus, species difference is obvious, not only for basal insulin secretion (paper 6), but also for glucose-induced insulin release (paper 7).

3. The β-Adrenoceptor and Insulin Secretion

β-Adrenoceptor stimulation has earlier been shown to promote insulin secretion in man, rats, dogs, and mice (Porte 1967b, Furman and Tayo, 1974, Kaneto et al. 1975, Lundquist and Ericson 1978). The β-adrenoceptors regulating insulin secretion are assumed to be of the β_2-type (Loubatières et al. 1971, Kaneto et al. 1975). The influences of the β-adrenoceptors on basal and stimulated insulin secretion and the proposed subdivision of the β-adrenoceptors were studied, in the present investigation, *in vivo* in the mouse and rat (papers 1, 8, 9, and 11).

It was demonstrated that at a low dose, the levó-rotatory isomer of propranolol depressed basal insulin levels (papers 1 and 9). Contrary, the racemic form of propranolol was without such an effect (paper 8), despite the fact that a higher dose was used. Obviously, the effective β-adrenoceptor blockade was lower for a comparable dose of the racemate than for the L-form, perhaps depending on receptor occupancy by the inactive D-propranolol. This might, in addition to dose and species differences, explain why in some studies no effect on basal insulin was seen after β-adrenoceptor blockade (Cerasi et al. 1972, Furman and Tayo, 1974).

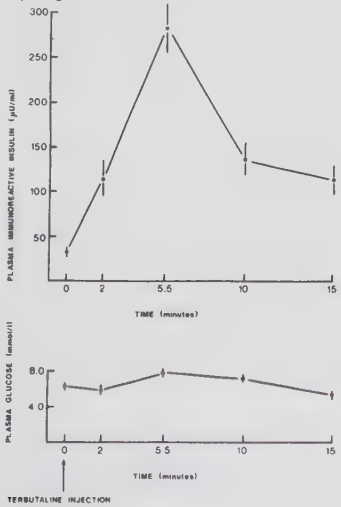
Thus, intact β-adrenoceptors seemed to be of importance for basal insulin secretion in the mouse (cf. Lundquist 1972a), as earlier has been found in man and baboons (Gagliardino et al. 1970, Goodner et al. 1973). Further, elimination of the sympatho-adrenal system lowered plasma insulin concentrations (papers 6 and 7). Consequently, it can be speculated that endogenous β-adrenoceptor stimulatory agents exist, and that these agents are of importance for the secretion of insulin. Phentolamine blocks the α-adrenoceptors and stimulates catecholamine synthesis (Dairman and Udenfriend 1970). Thus, phentolamine treatment can be regarded as a β-adrenoceptor stimulation, exerted by endogenous amines. Accordingly, it has been found in several species that phentolamine enhances plasma insulin concentrations (Frohman et al. 1967, Gagliardino et al. 1970. Lundquist 1972b, Furman and Tayo 1974, Smith et al. 1976, Lundquist and Ericson 1978, Järhult et al. 1979). This effect is blocked by propranolol (Furman and Tayo 1974, Lundquist and Ericson 1978).

It was shown in the present investigation (paper 6), that phentolamine enhanced insulin secretion in normal mice, and that this increase was diminished by chemical sympathectomy and adrenalectomy. Phentolamine was practically devoid of any insulin secretory effects in chemically sympathectomized-adrenalectomized mice. Hence, it is reasonable to assume that the effects of phentolamine are mediated by endogenous catecholamines, and that phentolamine has no direct effects on insulin secretion. Also in the rat, phentolamine enhanced insulin secretion, and the enhancement was abolished by 6-hydroxydopamine treatment and adrenalectomy (paper 10). After splanchnicotomy and adrenalectomy, the insulin secretory response to phentolamine was preserved; the response was probably mediated by catecholamines of remaining nerve terminals. These findings further strengthen the hypothesis, that catecholamines seem to have β-adrenoceptor stimulatory properties of importance for insulin secretion.

Administration of β-adrenoceptor agonists stimulate insulin secretion in man, rats, dogs, and mice (Porte 1967b, Loubatières et al. 1971, Furman and Tayo 1974, Kaneto et al. 1975, Lundquist and Ericson 1978). In the present study, the non-selective β-agonist IPNA as well as the selective β_2-agonist terbutaline were found to stimulate insulin secretion in the mouse (paper 8 and Fig. II); the effects were prevented by propranolol (papers 1 and 9). After propranolol pretreatment, terbutaline even depressed insulin secretion, which indicates that this β_2-agonist has α-adrenoceptor stimulatory properties as well. The time-course of the insulin secretory response to the β-adrenoceptor agonists was different from the time-course of the insulin response to glucose, carbachol and to various polypeptides, such as CCK-8, GIP, and glucagon (cf. paper 8, Figs II and III). β-Agonist-induced stimulation had a peak value of plasma insulin concentrations at a later time-point than stimulation by the other secretagogues. This might be due to different intracellular events after stimulation, or to transient inhibitory influences elicited by β-agonists. Such an indirect effect might be enhancement of noradrenaline release from adrenergic nerve terminals, a process which has been shown to be stimulated by β-adrenoceptors (Adler-Graschinsky and Langer 1975). The released noradrenaline might then inhibit direct effects of terbutaline, and diminish or delay the insulin secretory response. However, after chemical sympathectomy and adrenalectomy, when this possibility was prevented, the time course for terbutaline-induced insulin secretion was unaffected (Fig. VII).

Fig. VII

Changes in plasma concentrations of immunoreactive insulin and glucose following the i.v. injection of terbutaline (3.6 μmol/kg) in mice subjected to adrenalectomy 22 days and chemical sympathectomy by 6-hydroxydopamine (40 mg/kg) 8 days prior to the experiments. Mean ± SEM is shown. There were 10 animals in the group.



The selective β_1-agonist ITP had a much weaker insulin secretory response than L-IPNA and terbutaline, which is in agreement with the conclusions that β-adrenoceptors involved in insulin secretion *in vivo* are of the β_2--type (Loubatières et al. 1971). Earlier, however, some results, such as inhibition by selective β_1-adrenoceptor blocking drugs of L-IPNA-induced insulin secretion (Furman and Tayo 1974), have been reported, that are in conflict with this statement. This might be due to non-specific effects or incomplete selectivity of β_1-blocking drugs, or to participation of β_1-sensitive receptors in insulin secretion. In the present study, a variety of β-adrenoceptor blocking agents, selective and non-selective, were tested in their effects on basal, terbutaline- and glucose-induced insulin secretion (paper 8). It was concluded, that the subdivision of the β-adrenoceptors regulating insulin secretion *in vivo* in the mouse is not complete, though the receptors are mainly of the β_2-type.

Glucose-induced insulin secretion has been shown earlier to be partially dependent on intact β-adrenoceptors *in vivo* (Cerasi et al. 1972, Furman and Tayo 1974). This was confirmed in the present study (papers 8 and 9). The selective β_2-blocker ICI 118,551 and the non-selective β-blocker propranolol partially inhibited glucose-induced insulin secretion. L-Propranolol also inhibited the insulin secretion induced by CCK-8, CCK-39, and glucagon (papers 1 and 9). The interactions by L-propranolol with other stimulatory pathways than that induced by β-adrenoceptor agonists can be due to inhibition at the receptor level. In addition, after administration of propranolol and the secretagogues, intracellular events might compete. The suggestion that the effects of propranolol on insulin secretion can be ascribed to its membrane stabilizing effect (Myers and Hope-Gill 1979) was carefully studied by using, as controls, the dextro-rotatory form of propranolol, D-propranolol. This drug exerts identical membrane stabilizing activity as L-propranolol, but is devoid of β-adrenolytic action (Howe and Shanks 1966, Barrett and Cullum 1968). It was found, that D-propranolol was without effect on insulin secretion induced by glucose, glucagon, terbutaline (paper 9) or CCK-8 (data not shown). Thus, it is assumed that the β-adrenoceptors are necessary for the expression of full insulin secretory activity after stimulation with glucose, glucagon, terbutaline, and CCK-8.

Four additional aspects on β-adrenoceptor stimulated insulin secretion were studied in the present investigation. Firstly, the interactions between β-adrenoceptor stimulated insulin secretion and polypeptides were studied (papers 1, 2, 11, and Fig. VI). The results of these studies are discussed in chapter III. Secondly, it was demonstrated that intracellular accumulation of dopamine in the insulin cells diminishes L-IPNA-induced insulin secretion (paper 12). This is in agreement with an earlier study (Ericson et al. 1977). Thirdly, β-adrenoceptor stimulated insulin secretion was potentiated in hyperthyroid animals, and the insulin response was impaired after radiothyroidectomy (paper 13). This is in accordance with the assumption that the functional state of the β-adrenoceptors is partly regulated by the thyroid hormones (Banerjee and Kung 1977, Guidicelli 1978, Malbon et al. 1978). Fourthly, it was shown that methylatropine pretreatment induced a potentiated insulin release in response to terbutaline (paper 9). This might be due to intracellular changes after methylatropine treatment. Perhaps methylatropine treatment is followed by an enhanced responsiveness in the subsequent cAMP formation process induced by terbutaline (cf. Watanabe et al. 1978).

4. The Muscarinic Receptor and Insulin Secretion

Cholinergic influences are known to enhance insulin secretion in a variety of species (cf. Gerich and Lorenzi 1978, Edward and Bloom 1978). In the present investigation, cholinergic stimulation with carbachol enhanced

insulin secretion in a dose-dependent manner in vivo in the mouse (Fig. II). The maximal response was comparable to that induced by glucose (Fig. II), and the time-course in insulin secretion was similar as that brought about by glucose and several polypeptides (paper 8 and Fig. III). The influences on carbachol-induced insulin secretion of various polypeptides, chemical sympathectomy, adrenalectomy, autonomic blockade, intracellular dopamine, hyper- and hypothyroidism, have been investigated in the present study and are discussed in other chapters (papers 1, 2, 3, 4, 5, 7, 9, 12, and 13).

Atropine has been demonstrated earlier to inhibit cholinergically induced insulin secretion (Findlay et al. 1969, Kaneto and Kosaka 1974), but to be without effect on insulin secretion induced by intravenous glucose in man (Henderson et al. 1976). Arginine-induced insulin secretion has been found to be enhanced after atropine (Bloom et al. 1974a).

Atropine has not been found to have any effects on basal insulin secretion in man (Henderson et al. 1976). Recent studies in conscious calves, have revealed, however, a diminished basal insulin and an impaired insulin response to glucose after atropine (Bloom et al. 1978, Bloom and Edwards 1980). The influences of muscarinic blockade on insulin secretion induced to comparable niveau by different secretagogues were investigated in vivo in the mouse in the present study (paper 9).

A slight inhibition of basal insulin secretion was noted after treatment with methylatropine (papers 1 and 9). Methylatropine had no effect on the insulin secretory responses to glucose or glucagon. It inhibited the insulin response to carbachol, CCK-8 and CCK-39, and potentiated the response to terbutaline. Methylatropine does not, in contrast with atropine, cross the blood-brain barrier. Thus, the effects in this study illustrate the influences of the peripheral muscarinic receptors only. It is thus seen that the insulin secretory effects of CCK-8 and CCK-39 are dependent on intact muscarinic receptors.

After methylatropine treatment, the insulin secretory response to terbutaline was potentiated. Cholinergic stimulation has been shown to be associated with an increased formation of cGMP (George et al. 1970, Howell and Montague 1974). Cholinergic blockade may thus decrease cGMP, and this may lead to increased formation of cAMP, or increased affinity for β-agonists of the β-adrenoceptors (cf. Watanabe et al. 1978). If so, this might explain the increased insulin secretion after terbutaline in methylatropine-treated animals. However, in a recent report, no change in cGMP after cholinergic stimulation of pancreatic islets could be demonstrated (Gagerman et al. 1978).

5. Summary

Chemical sympathectomy, surgical splanchnicotomy, adrenalectomy, and blockade of β-adrenoceptors and muscarinic receptors induced alterations in basal and stimulated insulin secretion (papers 1, 6, 7, 8, 9, and 10). In the mouse, β-adrenoceptor and muscarinic blockade depressed basal insulin levels. Further, chemical sympathectomy and adrenalectomy were also followed by a decreased basal insulin secretion in the mouse. In the rat, chemical sympathectomy and surgical splanchnicotomy together with adrenalectomy elevated basal plasma insulin concentrations. Thus, species differences are observed between mouse and rat in this respect.

The influences on stimulated insulin secretion by manipulation with the sympatho-adrenal system in the mouse were highly dependent on the nature of the stimulus. This indicates that the secretagogues used, glucose, terbuta-

line, carbachol, and CCK-8, have different action mechanisms on insulin secretion *in vivo* (see Tables IV and V).

The β-adrenoceptors involved in insulin secretion are mainly of the β_2-type. β-Adrenoceptor stimulated insulin secretion is diminished by intracellular dopamine, by hypothyroidism and by various polypeptides such as CCK-8, CCK-39, secretin, and somatostatin. Furthermore, β-adrenoceptor induced insulin release is less sensitive to somatostatin than glucose-induced. In addition, insulin secretion stimulated by the β-adrenoceptor agonist terbutaline is enhanced after methylatropine pretreatment (papers 1, 2, 11, 12, 13).

The physiological importance of the neural influences on insulin secretion is not yet established. Transplanted and thus denervated islets have the capability to improve a diabetic state, which might indicate a less important role for the nerves (Ballinger and Lacy 1972). However, the precision control of insulin secretion is disturbed in such islets. Thus, denervated islets in the rat had a delayed onset of, and diminished magnitude in insulin secretion after oral glucose. This impairment was suggested to be due to the disturbed innervation, and associated with an abnormal glucose tolerance (Trimble et al. 1980).

Adrenergic nerves may be of most importance in stress, and cholinergic nerves after feeding (Edwards and Bloom 1978, Steffens 1980). How the various nervous systems interact and how they affect other insulin secretagogues, is not yet well known, but the present study has thrown some light on such interactions. Tables IV and V summarize the influences of manipulations with the sympatho-adrenal system and administration of methylatropine on basal and stimulated insulin secretion in the conscious mouse.

Table IV

Summary of the Effects on Insulin Secretion of the Autonomic Nervous System in the Mouse

Manipulation	Duration	Basal Insulin	Insulin secretion stimulated by β-agonist	glucose	carbachol	CCK-8
Chemical Sympathectomy	2 days	0	0	0	+	--
Chemical Sympathectomy	8 days	-	+	0	+	-
Chemical Sympathectomy	6 weeks	-	nt	+	nt	nt
Adrenalectomy	16 days	-	0	0	+	--
Adrenalectomy	22 days	-	0	+	+	0
Combined	short	-	0	+	++	--
Combined	long	--	-	++	+	0

+ indicates a different degree of stimulation, - indicates a different degree of inhibition, 0 indicates no effect and nt indicates not tested. Combined means the combined treatment of chemical sympathectomy and adrenalectomy (short indicates that chemical sympathectomy and adrenalectomy were performed 2 and 16 days before, and long 8 and 22 days before the experiments, respectively).

Table V

Summary of the Effects on Insulin Secretion of Autonomic Blockade in the Mouse

Blocker	Type	Basal Insulin	Insulin secretion stimulated by ß-agonist	glucose	carbachol	CCK-8	glucagon
L-propranolol	ß-adr.	--	--	-	0	--	--
Methylatropine	muscarinic	-	+	0	--	--	0
Butoxamine	$ß_2$-adr.	0	0	0			
ICI 118,551	$ß_2$-adr.	0	---	--			
Metoprolol	$ß_1$-adr.	(-)	-	(-)			
Pamatolol	$ß_1$-adr.	0	0	0			

+ indicates stimulation, - indicates a different degree of inhibition and 0 indicates no effect.

V. INFLUENCES OF INTRACELLULAR DOPAMINE AND THE THYROID STATE ON INSULIN SECRETION

Two additional aspects on the regulation of insulin secretion *in vivo* are considered in this summary; namely the influences of intracellular accumulation of dopamine in islet endocrine cells and of changes in thyroid activity (papers 12 and 13).

1. Intracellular Dopamine Accumulation

Insulin and glucagon cells in several species, such as the guinea pig, cat, dog and horse are known to contain the monoamines dopamine or serotonin (Falck and Hellman 1963, Cegrell 1968, Jacoby and Bryce 1978, Sundler et al. 1979). Also PP cells in certain species store monoamines intracellularly (Larsson et al. 1976a). Further, islet endocrine cells have the capability to take up and decarboxylate the amino acids L-DOPA and L-5-HTP to dopamine and serotonin, respectively (Cegrell 1968, Ekholm et al. 1971, Gylfe et al. 1973, Ericson et al. 1977, Pulido et al. 1978). Certain species, such as the rat and mouse, have no, by available histochemical methods, detectable amounts of amines in islet endocrine cells. However, insulin and glucagon cells have been shown to have the capability to take up and decarboxylate L-DOPA and L-5-HTP also in these species (Cegrell 1968). In islets, the monoamine degrading enzyme monoamine oxidase has been demonstrated (Feldman and Chapman 1974).

The intracellularly stored dopamine and serotonin, whether occurring naturally or accumulated after administration of L-DOPA or L-5-HTP, seem to be confined to the secretory granules (Jaim-Etcheverry and Zieher 1968, Ekholm et al. 1971, Gylfe et al. 1973, Ericson et al. 1977). Dopamine is located in the periphery of the granules (Ericson et al. 1977).

The function of the intracellular amines is not fully elucidated. Since they seem to be localized to secretory granules, it is reasonable to assume that they are of importance for storage or secretion of insulin and glucagon. No correlation seems, however, to exist between amine and hormone content and thus an influence on hormone storage seems less probable (Lundquist et al. 1975, cf. Sundler et al. 1979). Instead an effect on hormonal secretion might be the function of intracellular amines. In several reports such an effect has been demonstrated and intracellular amines are generally believed to exert inhibitory influences on insulin secretion (Lernmark 1971, Lundquist et al. 1971, Mahoney and Feldman 1977, Ericson et al. 1977, cf. Sundler et al. 1979). However, lack of effect of serotonin has also been reported (Pulido et al. 1978). Intracellular dopamine accumulation has earlier been shown to inhibit glucose-, glibenclamide- and L-IPNA-induced insulin secretion (Ericson et al. 1977).

In the present investigation, the effects of intracellular dopamine accumulation on stimulated insulin and glucagon secretion as well as on $^{45}Ca^{++}$-efflux from isolated islets were studied (paper 12 and Fig IV).

Dopamine accumulation was induced by the administration of L-DOPA and the monoamine oxidase inhibitor pargyline. L-DOPA and pargyline induced a marked accumulation of dopamine in islet endocrine cells. This was shown by fluorescence histochemistry, and by counting aliquots of homogenized islets after injection of radiolabelled L-DOPA or dopamine. Somatostatin cells seemed to have no capability to take up and decarboxylate L-DOPA. A more efficient cellular uptake in insulin and glucagon cells of L-DOPA than of dopamine was observed. Also, L-5-HTP was more efficiently taken up by the cells than serotonin. This is in agreement with results from several species (Cegrell 1968, Mahony and Feldman 1977). From *in vitro*

studies it has, however, been concluded that monoamines also have the capability to penetrate islet cell membranes (Hellman et al. 1972).

The insulin secretory experiments were performed 2 hours after administration of L-DOPA. Two hours after an injection of L-DOPA to mice, the amino acid is considered to be largely eliminated (Wurtman et al. 1970, cf. Sundler et al. 1979). Thus, effects on insulin and glucagon secretion at that time is assumed to be dependent on the intracellular accumulation of dopamine. It was found, that insulin secretion induced by L-IPNA or carbachol was inhibited after dopamine accumulation, whereas that induced by arginine was virtually unaffected. Basal insulin secretion and CCK-8--induced insulin secretion were depressed after an extended period of monoamine oxidase inhibition and L-DOPA-administration. This latter finding could partly be ascribed to the lowered plasma glucose levels (paper 12 and Fig. IV).

In previous studies, it has been shown that ß-adrenoceptor agonists, cholinergic agonists and arginine are efficient glucagon secretory agents (Iversen 1973a, 1973b, Kaneto and Kosaka 1974, Kaneto et al. 1975, Efendić et al. 1977, Pederson and Brown 1978). In the present study, the influence of intracellular dopamine on glucagon secretion induced by L-IPNA, carbachol or arginine was studied (paper 12). After intracellular accumulation of dopamine, the glucagon secretory response to L-IPNA was severely impaired. In contrast, glucagon secretion in response to carbachol and arginine was unaffected. Thus, when intracellular dopamine has an effect on glucagon secretion it is of an inhibitory nature. This is in contrast to extracellular dopamine, which stimulates glucagon secretion (Lorenzi et al. 1979).

The mechanism of action of intracellular amines is not known (cf. Sundler et al. 1979). Since the amines seem to be confined to secretory granules they may be released concomitantly with insulin (cf. Gylfe 1978), and thus subsequently stimulate extracellular receptors. However, since some intra- and extracellular effects of the amines seem to be non-parallel, this does not explain the mechanism of action of intracellular amines (cf. Lorenzi et al. 1979, paper 12). Another hypothesis is that the amines may bind to insulin and diminish its secretion (Barâth 1974). The amines have also been suggested to inhibit the activity of certain lysosomal enzymes, which are thought to be of importance for insulin secretion (cf. Lundquist 1971). A similar subcellular location of dopamine and a certain Ca^{++}-pool in the peripheral part of the secretory granules (Herman et al. 1973, Schäfer and Klöppel 1974, Ericson et al. 1977) suggested a possible interaction of dopamine with Ca^{++} metabolism (Ericson et al. 1977). The Ca^{+++}-pool might be of importance for glucose-induced insulin secretion (cf. Hellman et al. 1979). The effects of intracellular amines on Ca^{++}-metabolism is of interest for this latter hypothesis. Ca^{++} uptake by isolated islets has been shown to be diminished by serotonin (Lindström and Sehlin 1980).

In the present study, the effects of intracellularly accumulating dopamine on $^{45}Ca^{++}$-efflux from prelabelled isolated rat islets were investigated in a dynamic perifusion system (paper 12). It was found that L-DOPA--induced dopamine accumulation in islets subjected to monoamine oxidase inhibition increased the $^{45}Ca^{++}$-efflux. This effect was diminished by the decarboxylase inhibitor benserazide. Thus, the effect seemed to depend on dopamine. Whether the effect on $^{45}Ca^{++}$-efflux is due to membraneous effects leading to an increased Ca^{++}-Ca^{++}-exchange or to effects involving direct effects on secretory granular Ca^{++} is not yet known. However, an influence on islet Ca^{++}-metabolism by intracellular dopamine is indicated.

In a separate series of experiments (also presented in paper 12) the neural influences on the indigenous serotonin storage in guinea pig islets were investigated. It was found, that vagotomy decreased and chemical sympathectomy by 6-hydroxydopamine increased the intracellular content of serotonin. Thus, the autonomic nervous system seems to regulate the intracellular amine stores. It is not known whether these influences are of importance for the effects of the nerves on insulin or glucagon secretion.

In summary, L-DOPA injection concomitantly with pargyline treatment leads to a marked accumulation of dopamine in mouse insulin and glucagon cells in vivo. This is in contrast to treatment with dopamine itself. After dopamine accumulation, the insulin secretory responses to L-IPNA, carbachol and CCK-8 are inhibited, whereas the insulin response to arginine is unaffected. L-IPNA-induced glucagon secretion is inhibited, and glucagon release, stimulated by carbachol and arginine, unaffected by dopamine accumulation. Intracellular dopamine seems to stimulate the $^{45}Ca^{++}$-efflux from perifused prelabelled rat islets. Thus, intracellular dopamine has effects on both hormonal release from and Ca^{++}-metabolism in pancreatic islets.

2. Hyper- and Hypothyroidism

Clinically hyperthyroidism is often associated with disturbances in carbohydrate metabolism (Lamberg 1965). Alterations in insulin secretion have also been noted. However, the results are conflicting; insulin secretion after glucose or arginine stimulation has been shown to be enhanced, unchanged or diminished in hyperthyroidism (Andreani et al. 1970, Cavagnini et al. 1974, Andersen et al. 1977, Wajchenberg et al. 1978, Kabadi and Eisenstein 1980). The discrepancies may be ascribed to differences in the clinical expression of hyperthyroidism or to the duration of the disease. Administration of thyroxine for 11 days to rats resulted in diminished insulin secretory response to glucose, unaffected to glucagon and enhanced to β-adrenoceptor stimulation (Okajima and Ui 1978). Short-term administration of thyroxine to dogs resulted in inhibited glucose-induced insulin secretion (Renauld et al. 1971). This was, however, in contrast to long-term administration (Renauld et al. 1978).

In the present study (paper 13), the effects of long-term hyper- and hypothyroidism on stimulated insulin secretion were studied in conscious mice. Plasma glucose concentrations were depressed in hyperthyroid mice, and this was associated with decreased glycogen content in liver and muscle. In hyperthyroidism glucose turnover is enhanced (McCulloch et al. 1980). This might lead to exhausted glycogen reserves and subsequent lowering of plasma glucose. Also, triiodothyronine treatment has been shown to impair the glycogen synthetase activity in the liver (Orunesu et al. 1969).

Insulin secretory responses to the four chemically different secretagogues, glucose, terbutaline, carbachol, and CCK-8, were all enhanced in hyperthyroid mice and depressed in hypothyroid. Most affected was the insulin secretory response to β_2-adrenoceptor stimulation by terbutaline. This is of interest since the relation of β- to α-adrenoceptor activity has been suggested to be regulated by the thyroid hormones (Banerjee and Kung 1977, Giudicelli 1978, Malbon et al. 1978). Also glucose and CCK-8 seem to be dependent on β-adrenoceptors in their insulin secretory activity (papers 1, 8, and 9). The change in glucose and CCK-8 effects on insulin secretion in hypo- and hyperthyroidism might, in part, be explained by an effect on adrenoceptor activity of thyroid hormones. In addition, the thyroid hormones might have other effects which may modulate insulin sec-

retion or influence the islet cell mass. Thyroid hormones are known to modulate Ca^{++}-metabolism, protein synthesis and Na^{+}/K^{+} pump activity (cf. Sterling 1978). Irrespective of the mechanisms of action of the thyroid hormones, the four different secretagogues showed almost parallel changes in insulin secretory activity after alterations in the thyroid state.

3. Summary

Table VI summarizes the effects of intracellular dopamine accumulation and manipulations with the thyroid state on basal and stimulated insulin secretion. It is seen that when effects are altered, they are inhibited after dopamine accumulation and hypothyroidism, whereas they are potentiated after hyperthyroidism.

Table VI

Summary of the Effects on Insulin Secretion of Intracellular Dopamine and Changes in the Thyroid State in the Mouse

Manipulation	Plasma Glucose	Basal Insulin Secretion	Insulin secretion stimulated by Glucose	β-agonist	Carba-chol	CCK-8	Arginine
Dopamine accumulation	0	0	nt*	-	-	0	0
Dopamine accumulation and hypoglycemia	↓	-	nt*	--	--	--	0
Hyperthyroidism	↓	0**	++	++	+	+	nt
Hypothyroidism	0	0	-	--	--	-	nt

+ indicates a different degree of stimulation, ↓ indicates decrease, - indicates a different degree of inhibition, 0 indicates no effect, nt indicates not tested, * indicates not tested in this study but in an early study found to be moderately inhibited (Ericson et al. 1977), ** indicates enhanced in relation to plasma glucose levels.

VI. SUMMARY AND CONCLUSIONS

Various aspects on insulin secretion were studied *in vivo* in the mouse and rat, and the results have been discussed in this summary.

In the conscious mouse, insulin secretion induced by various polypeptides, β-adrenoceptor-stimulatory agents, glucose, and the cholinergic agonist carbachol were characterized with regard to dose-response and time-course relationships.

The effects of β-adrenoceptor blocking agents, intracellular dopamine accumulation, hyper- and hypothyroidism, chemical sympathectomy by 6-hydroxydopamine, adrenalectomy, and muscarinic blockade, on basal insulin levels and the insulin secretory response to chemically different secretagogues were, furthermore, investigated. Also, interactions between polypeptides and different secretagogues were studied.

Moreover, the effects of intracellular dopamine on stimulated glucagon secretion *in vivo* in the mouse and on $^{45}Ca^{++}$-efflux from perifused rat islets were investigated.

In the anaesthetized rat, the influences on insulin secretion of somatostatin, surgical splanchnicotomy, chemical sympathectomy, and α-adrenoceptor blockade were studied.

In addition, gastro-entero-pancreatic cellular distribution of various polypeptides, and morphological aspects of the adrenergic innervation of islets and intracellular amine storage, were studied in the mouse and rat. Lastly, the influences of vagotomy and chemical sympathectomy on indigenous intracellular serotonin were studied in guinea pig islets.

The following main conclusions were drawn:

1. Certain gut and islet polypeptides, such as CCK-8, CCK-39, GIP, and glucagon, stimulate insulin secretion in the basal state in the conscious mouse. The pattern of interaction with glucose-, carbachol- and L-IPNA-stimulated insulin secretion is characteristic for each peptide. The polypeptides have effects on stimulated insulin secretion at a lower dose level than on basal insulin secretion.

2. The adrenergic nerves and the adrenals exert an inhibitory tonus on basal insulin secretion in the rat, whereas in mice, the sympatho-adrenal system promotes basal insulin secretion. In addition, endogenous catecholamines have the capability to enhance insulin secretion. In the mouse, insulin secretion stimulated by glucose or the cholinergic agonist carbachol is enhanced after chemical sympathectomy and/or adrenalectomy, whereas that stimulated by CCK-8 is inhibited. Insulin secretion stimulated by the β_2-adrenoceptor agonist terbutaline is enhanced after chemical sympathectomy.

3. β-Adrenoceptor agonists stimulate insulin secretion in the mouse and rat. The β-adrenoceptors involved in insulin secretion are mainly of the β_2-type. Basal insulin secretion and the insulin secretory response to β_2-adrenoceptor stimulation, CCK-8, CCK-39, and glucagon, are notably dependent on intact β-adrenoceptors.

4. Somatostatin inhibits glucose-induced insulin secretion more potently than it inhibits the insulin secretory response to β-adrenoceptor stimulation in the mouse and rat. Moreover, in the mouse, somatostatin more potently inhibits cholinergically induced insulin secretion than it inhibits glucose-induced insulin secretion.

5. In the rat, the splanchnic nerves exert an inhibitory tonus on insulin secretion. Abolition of the tonus results in an enhancement of insulin secretion. This enhancement is prevented by β-adrenoceptor blockade.

6. Muscarinic blockade inhibits insulin secretion induced by cholinergic stimulation, CCK-8, and CCK-39 in the mouse. The insulin secretory response to glucose and glucagon is independent of muscarinic receptors. After muscarinic blockade, β_2-adrenoceptor stimulated insulin secretion is enhanced.

7. Intracellular dopamine inhibits L-IPNA- and carbachol-induced insulin secretion in the mouse, whereas it has no effect on that induced by arginine. Intracellular dopamine accumulation during an extended period of monoamine oxidase inhibition depresses plasma glucose concentrations. In such animals, L-IPNA-, carbahol-, and CCK-8-induced insulin secretion is severely impaired, whereas insulin secretion after arginine is preserved. Further, intracellular dopamine accumulation stimulates $^{45}Ca^{++}$-efflux from prelabelled rat islets. In addition, L-IPNA-stimulated glucagon secretion is depressed by intracellular dopamine in the mouse.

8. In guinea pig islets, vagotomy decreases and chemical sympathectomy by 6-hydroxydopamine increases the intracellular content of the monoamine serotonin.

9. Thyroid hormones influence insulin secretion in the mouse. The effects seem to be partially related to the functional balance between α- and β--adrenoceptor activity. Hyperthyroidism is associated with an increased response, and hypothyroidism with a decreased response to stimulation of insulin secretion by the chemically different secretagogues glucose, terbutaline, carbachol, and CCK-8.

VII. ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to

my teacher and co-author, Asisstant Professor Ingmar Lundquist, who introduced me to the field of experimental diabetology. He has supported me with invaluable help and constructive discussions throughout the study.

Professor Stephen Thesleff and Assistant Professor Claus Rerup, this department, who placed laboratory facilities at my disposal.

Professor Rolf Håkanson, this department, and Assistant Professors Pavo Hedner, Bengt Scherstén, Dept. of Medicine, Johannes Järhult, Dept. of Surgery, and Frank Sundler, Dept. of Histology, all University of Lund, for fruitful collaboration.

Mrs. Lena Kvist and Mr. Peter Okmark for invaluable and expert technical assistance, and to Mrs. Eva Björkbom for careful and expert secretarial assistance. Without their skilful work this study would not have been completed. For expert technical assistance I am also grateful to Miss Åsa Amilon and Mrs. Monica Radnell.

Dr. Per-Olof Bitzén, other colleagues and staff members at the Community Care Center, Dalby, for good collaboration during my clinical training.

my family, Iréne, Emma, and Jakob, for patience and support.

the Medical Faculty, University of Lund, Nordisk Insulinfond, Gentofte, Denmark, and to Eva och Oscar Ahrén Stiftelse, Stockholm, Sweden, for financial assistance.

VIII. REFERENCES

Adler-Graschinsky E, Langer SZ (1975) Possible role of a ß-adrenoceptor in the regulation of noradrenaline release by nerve stimulation through a positive feed-back mechanism. Brit J Pharmacol 53:43-50.

Ahrén B, Lundquist I (1980) Effects of glucagon and pentagastrin on glibenclamide-induced insulin release. Acta Physiol Scand 109:261-264.

Ahrén B, Ericsson M, Hedner P, Ingemansson S, Westgren U (1978) Somatostatin inhibits thyroid hormone secretion induced by exogenous TSH in man. J Clin Endocrinol Metab 47:1156-1159.

Ahrén B, Alumets J, Ericsson M, Fahrenkrug J, Fahrenkrug L, Håkanson R, Hedner P, Lorén I, Melander A, Rerup C, Sundler F (1980) VIP occurs in intrathyroidal nerves and stimulates thyroid hormone secretion. Nature 287:343-345.

Ajelis V, Björklund A, Falck B, Lindvall O, Lorén I, Walles B (1979) Application of the aluminium-formaldehyde (ALFA) histofluorescence method for demonstration of peripheral stores of catecholamines and indolamines in freeze-dried paraffin-embedded tissue, cryostat sections and whole-mounts. Histochemistry 65:1-15.

Alberti KGMM, Christensen JN, Christensen SE, Hansen AP, Iversen J, Lundbaek K, Seyer-Hansen K, Ørskov H (1973) Inhibition of insulin secretion by somatostatin. Lancet 2:1299-1301.

Alumets J, Håkanson R, O'Dorisio T, Sjölund K, Sundler F (1978) Is GIP a glucagon cell constituent? Histochemistry 58:253-257.

Alumets J, Ekelund M, El Munshid HA, Håkanson R, Lorén I, Sundler F (1979) Topography of somatostatin cells in the stomach of the rat: Possible functional significance. Cell Tiss Res 202:177-188.

Andersen OO, Friis T, Ottesen B (1977) Glucose tolerance and insulin secretion in hyperthyroidism. Acta Endocrinol Kbh 84:576-587.

Andreani D, Menzinger G, Fallucca F, Aliberti G, Tamburrano G, Cassano C (1970) Insulin levels in thyrotoxicosis and primary myxoedema: Response to intravenous glucose and glucagon. Diabetologia 6:1-7.

Banerjee SP, Kung LS (1977) ß-Adrenergic receptors in rat heart: Effects of thyroidectomy. Europ J Pharmacol 43:207-208.

Ballinger WF, Lacy PE (1972) Transplantation of intact pancreatic islets in rats. Surgery 72:175-186.

Banting FG, Best CH (1922) The internal secretion of the pancreas. J Lab Clin Med 7:251-266.

Baráth P (1974) New aspects of the role of serotonin in the insulin release mechanism. Acta Diabetol Lat 11:315-329.

Barden N, Lavoie M, Dupont A, Cotê J, Cotê JP (1977) Stimulation of glucagon release by addition of antisomatostatin serum to islets of Langerhans in vitro. Endocrinology 101:635-638.

Barrett AM, Cullum VA (1968) The biological properties of the optical isomers of propranolol and their effects on cardiac arrythmias. Brit J Pharmacol 34:43-55.

Basabe J, Cresto JC, Aparicio N (1977) Studies on the mode of action of somatostatin on insulin secretion. Endocrinology 101:1436-1443.

Baum J, Simons BE, Unger RH, Madison LL (1962) Localization of glucagon in the alpha cells in the pancreatic islets by immunofluorescent technics. Diabetes 11:371-374.

Bayliss WM, Starling EH (1902) The mechanism of pancreatic secretion. J Physiol 28:325-353.

Bergman RN, Miller RE (1973) Direct enhancement of insulin secretion by vagal stimulation of the isolated pancreas. Am J Physiol 225:481-486.

Bhathena SJ, Perrino PV, Voyles NR, Smith SS, Wilkins SD, Coy DH, Schally AV, Recant L (1976) Reversal of somatostatin inhibition of insulin and glucagon secretion. Diabetes 25:1031-1040.

Bishop AE, Polak JM, Green IC, Bryant MG, Bloom SR (1980) The location of VIP in the pancreas of man and rat. Diabetologia 18:73-78.
Blackburn AM, Bloom SR (1979) A radioimmunoassay for neurotensin in human plasma. J Endocrinol 83:175-181.
Blackburn AM, Bloom SR, Edwards AV (1980) Pancreatic endocrine responses to exogenous neurotensin in the conscious calf. J Physiol in press.
Bloom SR, Edwards AV (1975) The release of pancreatic glucagon and inhibition of insulin release in response to stimulation of the sympathetic innervation. J Physiol 253:157-173.
Bloom SR, Edwards AV (1978) Certain pharmacological characteristics of the release of pancreatic glucagon in response to stimulation of the splanchnic nerves. J Physiol 280:25-35.
Bloom SR, Edwards AV (1980) The role of the parasympathetic system in the control of insulin release. Diabetologia 19:258-259.
Bloom SR, Vaughan NJA, Russel RCG (1974a) Vagal control of glucagon release in man. Lancet 2:546-549.
Bloom SR, Edwards AV, Vaughan NJA (1974b) The role of the autonomic innervation in the control of glucagon release during hypoglycaemia in the calf. J Physiol 236:611-623.
Bloom SR, Edwards AV, Hardy RN (1978) The role of the autonomic nervous system in the control of glucagon, insulin and pancreatic polypeptide release from the pancreas. J Physiol 280:9-23.
Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive growth hormone. Science 179:77-79.
Bromer WW, Sinn LG, Staub A, Behrens OK, Diller ER, Bird HL (1957) The amino acid sequence of glucagon. V. Localization of amide groups, acid degradation studies, and summary of sequential evidence. J Am Chem Soc 79:2807-2810.
Brown JC, Dryburgh JR (1971) A gastric inhibitory polypeptide. II. The complete amino acid sequence. Can J Biochem 49:867-872.
Brown JC, Dryburgh JR, Ross SA, Dupré J (1975) Identification and actions of gastric inhibitory polypeptide. Rec Progr Horm Res 31:487-526.
Brown M, Vale W (1976) Effects of neurotensin and substance P on plasma insulin, glucagon and glucose levels. Endocrinology 98:819-822.
Bruss ML, Black AL (1978) Enzymatic microdetermination of glycogen. Analyt Biochem 84:309-312.
Budillon G, Mazzacca G, Squame G (1973) Failure of endogenous gastrin release to affect serum insulin. Digestion 8:201-207.
Burr IM, Jackson A, Culbert S, Sharp R, Felts P, Olson W (1974) Glucose intolerance and impaired insulin release following 6-hydroxydopamine administration to intact rats. Endocrinology 94:1072-1076.
Burr IM, Slonim AE, Burke V, Fletcher T (1976) Extracellular calcium and adrenergic and cholinergic effects on islet ß-cell function. Am J Physiol 231:1246-1249.
Cahill Jr GF (1971) Physiology of insulin in man. Diabetes 20:785-799
Cahill Jr GF (1976) Insulin and glucagon. In: Parsons JA (ed) Peptide Hormones. Macmillan, London. pp. 85-100.
v Campenhout E (1927) Contributions a l'étude de l'histogenèse du pancréas chez quelque mammifères. Les complex sympathetico-insulaires. Arch Biol Liege 37:121-171.
Carraway R, Leeman SE (1973) The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. J Biol Chem 248:6854-6861.
Carraway R, Leeman SE (1975) The amino acid sequence of a hypothalamic peptide, neurotensin. J Biol Chem 250:1907-1911.
Cataland S, Crockett SE, Brown JC, Mazzaferri EL (1974) Gastric inhibitory polypeptide (GIP): Stimulation by oral glucose in normal man. J Clin Endocrinol Metab 39:223-228.

Cavagnini F, Peracchi M, Raggi U, Bana R, Pontiroli AE, Malinverni A, Pinto M (1974) Impairment of growth hormone and insulin secretion in hyperthyroidism. Europ J Clin Invest 4:71-77.
Cegrell L (1968) The occurrence of biogenic monoamines in the mammalian endocrine pancreas. Acta Physiol Scand Suppl 314:1-60.
Cerasi E, Luft R (1967) The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. Acta Endocrinol Kbh 55: 278-304.
Cerasi E, Luft R, Efendić S (1972) Effect of adrenergic blocking agents on insulin response to glucose infusion in man. Acta Endocrinol Kbh 69:335-346.
Chey WY, Kim MS, Lee KY (1979) Influence of the vagus nerve on release and action of secretin in dog. J Physiol 293:435-446.
Chideckel EW, Palmer J, Koerker DJ, Ensinck J, Davidson MB, Goodner CJ (1975) Somatostatin blockade of acute and chronic stimuli of the endocrine pancreas and the consequences of this blockade on glucose homeostasis. J Clin Invest 55:754-762.
Chisholm DJ, Young JD, Lazarus L (1969) The gastrointestinal stimulus to insulin release. I Secretin. J Clin Invest 48:1453-1460.
Claro A, Grill V, Efendić S, Luft R (1977) Studies on the mechanisms of somatostatin action on insulin release. IV. Effect of somatostatin on cyclic AMP levels and phosphodiesterase activity in isolated rat pancreatic islets. Acta Endocrinol Kbh 85:379-388.
Colwell AR, Zuckerman L (1970) Regulation of insulin release by pancreatic glucagon. Diabetes 19:429-437.
Coons AH, Leduc EM, Connolly JM (1955) Studies on antibody production. I. A method for the histochemical demonstration of specific antibodies and its application to a study of the hyperimmune rabbit. J Exp Med 102:49-59.
Coupland RE (1958) The innervation of the pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. J Anat 92:143-149.
Creutzfeldt W (1979) The incretin concept today. Diabetologia 16:75-85.
Dairman W, Udenfriend S (1970) Effect of ganglionic blocking agents on the increased synthesis of catecholamines resulting from α-adrenergic blockade or exposure to cold. Biochem Pharmacol 19:979-984.
Deckert T, Birk Lauridsen U, Nistrup Madsen S, Mogensen P (1972) Insulin response to glucose, tolbutamide, secretin, and isoprenaline in maturity-onset diabetes mellitus. Dan Med Bull 19:222-226.
Dockray GJ (1977) Molecular evolution of gut hormones: Application of comparative studies on the regulation of digestion. Gastroenterology 72: 344-358.
Dockray GJ, Best L, Taylor IL (1977) Immunochemical characterization of gastrin in pancreatic islets of normal and genetically obese mice. J Endocrinol 72:143-151.
Dolais-Kitabgi J, Freychet P (1979) Neurotensin inhibits glucose but not glucagon-induced insulin and somatostatin release in isolated islets. Diabete & Metab 5:275-277.
Dolais-Kitabgi J, Kitabgi P, Brazeau P, Freychet P (1979) Effect of neurotensin on insulin, glucagon, and somatostatin release from isolated pancreatic islets. Endocrinology 105:256-260.
Dubois MP (1975) Immunoreactive somatostatin is present in discrete cells of the endocrine pancreas. Proc Natl Acad Sci USA 72:1340-1343.
Dupré J, Curtis JD, Unger RH, Waddell RW, Beck JC (1969) Effects of secretin, pancreozymin or gastrin on the response of the endocrine pancreas to administration of glucose or arginine in man. J Clin Invest 48:745-757.
Dupré J, Ross SA, Watson D, Brown JC (1973) Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 37:826-828.

Dupré J, Chisholm, DJ, McDonald TJ, Rabinovitch A (1975) Effects of secretin on insulin secretion and glucose tolerance. Can J Physiol Pharmacol 53:1115-1121.
Dupré J, Greenidge N, McDonald TJ, Ross SA, Rubinstein D (1976) Inhibition of actions of glucagon in adipocytes by gastric inhibitory polypeptide. Metabolism 25:1197-1199.
Ebert R, Illmer K, Creutzfeldt W (1979) Release of gastric inhibitory polypeptide (GIP) by intraduodenal acidification in rats and humans and abolishment of the incretin effect of acid by GIP-antiserum in rats. Gastroenterology 76:515-523.
Edkins JS (1905) Chemical mechanism of gastrin secretion. Proc Roy Soc Med 76:376.
Edwards AV, Bloom SR (1978) Nervous control of pancreatic hormones. In: Bloom SR (ed) Gut Hormones, Churchill Livingstone, Edinburgh, pp. 394-405.
Efendić S, Luft R (1975) Studies on the mechanism of somatostatin action on insulin release in man. I. Effect of blockade of α-adrenergic receptors. Acta Endocrinol Kbh 78:516-523.
Efendić S, Luft R, Claro A (1976) Studies on the mechanism of somatostatin action on insulin release in man. II. Comparison of the effects of somatostatin on insulin release induced by glucose, glucagon and tolbutamide. Acta Endocrinol Kbh 81:743-752.
Efendić S, Luft R, Pernow B (1977) Effect of substance P on arginine-induced insulin and glucagon release from the isolated perfused rat pancreas. In: v Euler US, Pernow B (eds) Substance P. Raven Press, New York, pp. 241-245.
Efendić S, Luft R, Cerasi E (1978a). Quantitative determination of the interaction between epinephrine and various insulin releasers in man. Diabetes 27:319-326.
Efendić S, Lins PE, Luft R (1978b) Somatostatin and insulin secretion. Metabolism 27:1275-1281.
Ekholm R, Ericson LE, Lundquist I (1971) Monoamines in the pancreatic islets of the mouse. Subcellular localization of 5-hydroxytryptamine by electron microscopic autoradiography. Diabetologia 7:339-348.
Elahi D, Andersen DK, Brown JC, Debas HT, Hershcopf RJ, Raizes GS, Tobin JD, Andres R (1979) Pancreatic α- and β-cell responses to GIP infusion in normal man. Am J Physiol 237:E185-E191.
Elrick H, Stimmler L, Hlad Jr CJ, Arai Y (1964) Plasma insulin responses to oral and intravenous glucose administration. J Clin Endocrinol Metab 24:1076-1082.
Enk B (1976) Secretin induced insulin response. II. Dose-response relation. Acta Endocrinol Kbh 82:312-317.
Ensinck JW, Williams RH (1974) Disorders causing hypoglycemia. In: Williams RH (ed) Textbook of endocrinology, 5th ed, Saunders Company, Philadelphia, London, Toronto, pp. 627-659.
Ericson LE, Håkanson R, Lundquist I (1977) Accumulation of dopamine in mouse pancreatic B-cells following injection of L-DOPA. Localization to secretory granules and inhibition of insulin secretion. Diabetologia 13:117-124.
von Euler US, Gaddum HJ (1931) An unidentified depressor substance in certain tissue extracts. J Physiol 72:74-87.
Fahrenkrug (1979) Vasoactive intestinal polypeptide: Measurement, distribution and putative neurotransmitter function. Digestion 19:149-169.
Fahrenkrug J, Schaffalitzky de Muckadell OB, Kühl C (1978) Effect of secretin on basal- and glucose-stimulated insulin secretion in man. Diabetologia 14:229-234.
Falck B, Hellman B (1963) Evidence for the presence of biogenic amines in pancreatic islets. Experientia 19:139-140.

Falck B, Hillarp NÅ, Thieme G, Torp A (1962) Fluorescence of catecholamines and related compounds condensed with formaldehyde. J Histochem Cytochem 10:348-354.
Feldman JM, Chapman BS (1974) Mechanisms of catecholamine inactivation by the endocrine pancreas, Diabetes 23:754-762.
Feldman JM, Lebovitz HE (1973) Role of pancreatic monoamines in the impaired insulin secretion of the fasting state. Endocrinology 92:1469-1474.
Findlay JA, Gill JR, Lever JD, Randle PJ, Spriggs TLB (1969) Increased insulin output following stimulation of the vagal supply to the perfused rabbit pancreas. J Anat 104:580.
Floyd Jr JC, Vinik AI, Glaser B, Fajans SS, Pek S (1980) Pancreatic polypeptide. In: Waldhäusl WK (ed) Diabetes 1979, Excerpta Medica, Amsterdam Oxford Princeton pp. 490-495.
Frame CM, Davidson MB, Studevant RAL (1975) Effects of the octapeptide of cholecystokinin on insulin and glucagon secretion in the dog. Endocrinology 97:549-553.
Frankel BJ, Imagawa WT, O'Connor MDL, Lundquist I, Kromhout JA, Fanska RE, Grodsky GM (1978) Glucose-stimulated ^{45}Calcium efflux from isolated rat pancreatic islets. J Clin Invest 62:525-531.
Frohman LA, Ezdinli EZ, Javid R (1967) Effect of vagotomy and vagal stimulation on insulin secretion. Diabetes 16:443-448.
Furman BL, Tayo FM (1974) Effect of some β-adrenoceptor blocking drugs on insulin secretion in the rat. J Pharm Pharmacol 26:512-517.
Gagerman E, Idahl LÅ, Meissner HP, Täljedal IB (1978) Insulin release, cGMP, cAMP, and membrane potential in acetylcholine-stimulated islets. Am J Physiol 235:E493-E500.
Gagliardino JJ, Bellone CF, Doria I, Sánchez JJ, Pereyra V (1970) Adrenergic regulation of basal serum glucose, NEFA and insulin levels. Horm Metab Res 2:318-322.
George W, Polson J, O'Toole A, Godlberg N (1970) Elevation of guanosine 3',5'-cyclic phosphate in rat heart after perfusion with acetyl choline. Proc Natl Acad Sci USA 66:398-403.
Gerich JE, Lorenzi M (1978) The role of the autonomic nervous system and somatostatin in the control of insulin and glucagon secretion. In: Ganong WF, Martini L (eds) Frontiers in Neuroendocrinology, vol 5, Raven Press, New York, pp. 265-288,
Gerich JE, Lovinger R, Grodsky GM (1975) Inhibition by somatostatin of glucagon and insulin release from the perfused rat pancreas in response to arginine, isoproterenol and theophylline: Evidence for a preferential effect on glucagon secretion. Endocrinology 96:749-754.
Giudicelli Y (1978) Thyroid hormone modulation of the number of β-adrenergic receptors in rat fat-cell membranes. Biochem J 176:1007-1010.
Goldberg NJ, Wingert TD, Levin, SR, Adachi RI (1980) Augmentation of insulin secretion by a non-nutrient drink. Gastroenterology 78:1458-1462.
Goodner CJ, Koerker DJ, Werrbach JH, Toivola P, Gale CC (1973) Adrenergic regulation of lipolysis and insulin secretion in the fasted baboon. Am J Physiol 224:534-539.
Grodsky GM, Bennett LL, Smith DF, Schmid FG (1967) Effect of pulse administration of glucose or glucagon on insulin secretion in vitro. Metabolism 16:222-233.
Grodsky GM, Lundquist I, Fanska R, Pictet R (1977) Interrelationship of calcium and somatostatin in the secretion of insulin and glucagon. In: Foa PP, Bajaj JS, Foa NL (eds) Glucagon: Its role in physiology and clinical medicine, Springer Verlag, New York Heidelberg Berlin, pp. 215-230.
Grube D, Forssmann WG (1979) Morphology and function of the entero-endocrine cells. Horm Metab Res 11:589-606.

Grube D, Maier V, Raptis S, Schlegel W (1978) Immunoreactivity of the endocrine pancreas. Evidence for the presence of cholecystokinin-pancreozymin within the A-cell. Histochemistry 56:13-35.
Gutman RA, Fink G, Voyles N, Selawry H, Penhos JC, Lepp A, Recant L (1973) Specific biologic effects of intestinal glucagon-like materials. J Clin Invest 52:1165-1175.
Gylfe E (1978) Association between 5-hydroxytryptamine release and insulin secretion. J Endocrinol 78:239-248.
Gylfe E, Hellman B, Sehlin J, Täljedal IB (1973) Amino acid conversion into 5-hydroxytryptamine in pancreatic ß-cells. Endocrinology 93:932--937.
Haeusler G, Haefely W, Heurlimann A (1969) Zum Mechanismus der adrenerg blockierenden Wirkung von Bretylium. Naunyn-Schmiedebergs Arch Pharmak Exp Path 264:241-243.
Harper AA, Raper HS (1943) Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. J Physiol 102:115-125.
Hazelwood RL, Turner SD, Kimmel JR, Pollock HG (1973) Spectrum effects of a new polypeptide (third hormone?) isolated from the chicken pancreas. Gen Comp Endocrinol 21:485-497.
Hedeskov CJ (1980) Mechanism of glucose-induced insulin secretion. Physiol Rev 60:442-509.
Heding LG (1966) A simplified radioimmunoassay method. In: Donato L, Milhaud G, Sirchis J (eds). Labelled proteins in tracer studies. Euratom, Brussels, pp. 345-350.
Heding LG (1971) Radioimmunological determination of pancreatic and gut glucagon in plasma. Diabetologia 7:10-19.
Hedner P, Persson G, Ursing D (1975) Insulin release in fasting man induced by impure but not by pure preparations of cholecystokinin. Acta Med Scand 197:109-112.
Hellman B, Lernmark Å, Sehlin J, Täljedal IB (1972) Transport and storage of 5-hydroxytryptamine in pancreatic ß-cells. Biochem Pharmacol 21: 695-706.
Hellman B, Andersson T, Berggren PO, Flatt P, Gylfe E, Kohnert KD (1979) The role of calcium in insulin secretion. In: Dumont J, Nunez J (eds) Hormones and cell regulation, vol III, Elsevier/North Holland Biomedical Press, Amsterdam, pp. 69-96.
Helmstaedter V, Feurle GE, Forssmann WG (1977) Ultrastructural identification of a new cell type - the N-cell as the source of neurotensin in the gut mucosa. Cell Tiss Res 184:445-452.
Henderson J, Jefferys DB, Jones RH, Stanley D (1976) The effect of atropine on the insulin release caused by oral and intravenous glucose in human subjects. Acta Endocrinol Kbh 83:772-780.
Herman L, Sato T, Hales CN (1973) The electron microscopic localization of cations to pancreatic islets of Langerhans and their possible role in insulin secretion. J Ultrastruct Res 42:298-311.
Hermansen K (1980) Effects of substance P and other peptides on the release of somatostatin, insulin and glucagon in vitro. Endocrinology 107:256--261.
Hindberg I, Enk B, Persson I (1970) Insulin stimulation by secretin in diabetics. Effects of repreated and of varied doses. Horm Metab Res 2:131-134.
Hökfelt T, Efendić S, Hellerström C, Johansson O, Luft R, Arimura A (1975a) Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A_1-cells of the pancreatic islets and to the hypothalamus. Acta Endocrinol Kbh 80: suppl 200:1-41.

Hökfelt T, Kjellert JO, Nilsson G, Pernow B (1975b) Substance P: Localization in the central nervous system and in some primary sensory neurons. Science 190:889-890.
Holst JJ (1978) Extrapancreatic glucagons. Digestion 17:168-190.
Holst JJ (1980) Evidence that glicentin contains the entire sequence of glucagon. Biochem J 187:337-343.
Hornnes PJ, Kühl C, Holst JJ, Lauritsen KB, Rehfeld JF, Schwartz TW (1980) Simultaneous recording of the gastro-entero-pancreatic hormonal peptide response to food in man. Metabolism 29:777-779.
Howe R, Shanks RG (1966) Optical isomers of propranolol. Nature 210:1336-1338.
Howell SL, Montague W (1974) Regulation of guanylate cyclase in guinea-pig islets of Langerhans. Biochem J 142:379-384.
Ipp E, Dobbs RE, Harris V, Arimura A, Vale W, Unger RH (1977a) The effects of gastrin, gastric inhibitory polypeptide, secretin, and the octapeptide of cholecystokinin upon immunoreactive somatostatin release by the perfused canine pancreas. J Clin Invest 60:1216-1219.
Ipp E, Dobbs RE, Arimura A, Vale W, Harris V, Unger RH (1977b) Release of immunoreactive somatostatin from the pancreas in response to glucose, amino acids, pancreozymin-cholecystokinin, and tolbutamide. J Clin Invest 60:760-765.
Ishida T (1977) Stimulatory effect of neurotensin on insulin and gastrin secretion in dogs. Endocrinol Japon 24:335-342.
Iversen J (1973a) Adrenergic receptors and the secretion of glucagon and insulin from the isolated, perfused canine pancreas. J Clin Invest 52:2102-2116.
Iversen J (1973b) Effect of acetyl choline on the secretion of glucagon and insulin from the isolated, perfused canine pancreas. Diabetes 22:381-387.
Iversen J, Hermansen K (1980) Characterization of the inhibitory effect of somatostatin upon insulin and glucagon release in the isolated perfused canine pancreas: Evidence of interaction with calcium. Metabolism 29:151-160.
Ivy AC, Oldberg E (1928) A hormone mechanism for gall-bladder contraction and evacuation. Am J Physiol 86:599-613.
Jacobsen H, Demandt A, Moody AJ, Sundby F (1977) Sequence analysis of porcine gut GLI-1. Biochim Biophys Acta 493:452-459.
Jacoby JH, Bryce GF (1978) Monoaminergic modulation of pancreatic endocrine secretion. Gen Pharmacol 9:411-419.
Jaim-Etcheverry G, Zieher LM (1968) Electron microscopic cytochemistry of 5-hydroxytryptamine (5-HT) in the beta cells of guinea pig endocrine pancreas. Endocrinology 83:917-923.
Järhult J, Holst JJ (1978) Reflex adrenergic control of endocrine pancreas evoked by unloading of carotid baroreceptors in cats. Acta Physiol Scand 104:188-202.
Järhult J, Ahrén B, Ericsson M, Holst J (1978) Failure of somatostatin to eliminate the glucagon release induced by baroreceptor unloading in the cat. Acta Physiol Scand 103:478-480.
Järhult J, Ahrén B, Lundquist I (1979) Inhibitory effect of somatostatin on insulin secretion during α-adrenergic blockade in three different species. Acta Endocrinol Kbh 92:166-173.
Järhult J, Hellstrand P, Sundler F (1980) Immunohistochemical localization and vascular effects of vasoactive intestinal polypeptide in skeletal muscle of the cat. Cell Tiss Res 207:55-64.
Kabadi UM, Eisenstein AB (1980) Glucose intolerance in hyperthyroidism: Role of glucagon. J Clin Endocrinol 50:392-396.
Kaneto A, Kosaka K (1974) Stimulation of glucagon and insulin secretion by acetylcholine infused intrapancreatically. Endocrinology 95:676-681.

Kaneto A, Miki E, Kosaka K (1974) Effects of vagal stimulation on glucagon and insulin secretion. Endocrinology 95:1005-1010.
Kaneto A, Miki E, Koasaka K (1975) Effect of beta and beta$_2$ adrenoreceptor stimulants infused intrapancreatically on glucagon and insulin secretion. Endocrinology 97:1166-1173.
Kaneto A, Kaneko T, Kajinuma H, Kosaka K (1977) Effect of vasoactive intestinal polypeptide infused intrapancreatically on glucagon and insulin secretion. Metabolism 26:781-786.
Kaneto A, Kajinuma H, Kaneko T, Kosaka K (1978a) Effect of phentolamine on the somatostatin-induced inhibition of glucagon and insulin secretion. Metabolism 27:901-910.
Kaneto A, Kaneko T, Kajinuma H, Kosaka K (1978b) Effects of substance P and neurotensin infused intrapancreatically on glucagon and insulin secretion. Endocrinology 102:393-401.
Kikuchi M, Kuzuya T, Ide T (1971) Plasma insulin response to intravenous administration of tetragastrin (C-terminal tetrapeptide amide of gastrin) in man. Metabolism 20:433-445.
Kimmel JR, Pollock HG, Hazelwood R (1968) Isolation and characterization of chicken insulin. Endocrinology 83:1323-1336.
Kimmel JR, Hayden LJ, Pollock HG (1975) Isolation and characterization of a new pancreatic polypeptide hormone. J Biol Chem 250:9369-9376.
Kobayashi S, Fujita T (1969) Fine structure of mammalian and avian pancreatic islets with special reference to D cells and nervous elements. Z Zellforsch 100:340-363.
Koerker DJ, Ruch W, Chideckel E, Palmer J, Goodner CJ, Ensinck J, Gale CC (1974) Somatostatin: Hypothalamic inhibitor of the endocrine pancreas. Science 184:482-484.
Kostrzewa RM, Jacobowitz DM (1974) Pharmacological actions of 6-hydroxydopamine. Pharmacol Rev 26:199-288.
Kuo WN, Hodgins DS, Kuo JF (1973) Adenylate cyclase in islets of Langerhans. Isolation of islets and regulation of adenylate cyclase activity by various hormones and agents. J Biol Chem 248:2705-2711.
LaBarre J, Still EU (1930) Studies on the physiology of secretin. III. Further studies on the effects of secretin on the blood sugar. Am J Physiol 91:649-653.
Lamberg BA (1965) Glucose metabolism in thyroid disease. Acta Med Scand 178:351-362.
Langerhans P (1869) Beiträge zur mikroskopischen Anatomie der Bauspeicheldrüse. Inaugural-Dissertation. Friedrich-Wilhelms-Universität, Berlin, pp. 1-32.
Larsson LI (1979a) Peptides of the gastrin cell. In: Rehfeld JF, Amdrup E (eds) Gastrins and the vagus. Academic Press, London New York San Francisco, pp 5-14.
Larsson LI (1979b) Innervation of the pancreas by substance P, enkephalin, vasoactive intestinal polypeptide and gastrin/CCK immunoreactive nerves. J Histochem Cytochem 27:1283-1284.
Larsson LI (1980) Gastrointestinal cells producing endocrine, neurocrine and paracrine messengers. Clin Gastroenterol 9:485-516.
Larsson LI, Rehfeld JF (1979) Peptidergic and adrenergic innervation of pancreatic ganglia. Scand J Gastroenterol 14:433-437.
Larsson LI, Holst J, Håkanson R, Sundler F (1975) Distribution and properties of glucagon immunoreactivity in the digestive tract of various mammals: An immunohistochemical and immunochemical study. Histochemistry 44:281-290.
Larsson LI, Sundler F, Håkanson R (1976a) Pancreatic polypeptide - a postulated new hormone: Identification of its cellular storage site by light and electron microscopic immunocytochemistry. Diabetologia 12:211-226.

Larsson LI, Rehfeld JF, Sundler F, Håkanson R (1976b) Pancreatic gastrin in foetal and neonatal rats. Nature 262:609-610.
Larsson LI, Fahrenkrug J, Schaffalitzky de Muckadell O, Sundler F, Håkanson R, Rehfeld JF (1976c) Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral neurons. Proc Natl Acad Sci USA 73:3197-3200.
Larsson LI, Sundler F, Alumets J, Håkanson R, Schaffalitzky de Muckadell OB, Fahrenkrug J (1977) Distribution, ontogeny and ultrastructure of the mammalian secretin cell. Cell Tiss Res 181:361-368.
Larsson LI, Goltermann N, de Magistris L, Rehfeld JF, Schwartz TW (1979) Somatostatin cell processes as pathways for paracrine secretion. Science 205:1393-1395.
Lawrence TS, Beers WH, Gilula NB (1978) Transmission of hormonal stimulation by cell-to-cell communication. Nature 272:501-506.
Lerner RL (1977) The augmentation effect of secretin on the insulin responses to known stimuli: Specificity for glucose. J Clin Endocrinol Metab 45:1-9.
Lernmark Å (1971) The significance of 5-hydroxytryptamine for insulin secretion in the mouse. Horm Metab Res 3:305-309.
Levin SR, Pehlevanian MZ, Lavee AE, Adachi RI (1979) Secretion of an insulinotropic factor from isolated, perfused rat intestine. Am J Physiol 236:E710-E720.
Lin TM, Chance R, Evans D (1973) Stimulatory and inhibitory actions of bovine pancreatic peptide (BPP) on gastric and pancreatic secretions of dogs. Gastroenterology 64:865.
Lindkaer Jensen S, Fahrenkrug J, Holst JJ, Vagn Nielsen O, Schaffalitzky de Muckadell OB (1978) Secretory effects of vasoactive intestinal polypeptide (VIP) on the isolated perfused porcine pancreas. Am J Physiol 235:E387-E391.
Lindkaer Jensen S, Rehfeld JF, Holst JJ, Fahrenkrug J, Vagn Nielsen O, Schaffalitzky de Muckadell OB (1980) Secretory effects of gastrins on isolated perfused porcine pancreas. Am J Physiol 238:E186-E192.
Lindström P, Sehlin J (1980) Tryptaminergic modifications of islet $^{45}Ca^{2+}$ fluxes and insulin secretion in vitro. Diabetologia 19:294-295.
Lomsky R, Langr F, Vortel V (1969) Immunohistochemical demonstration of gastrin in mammalian islets of Langerhans. Nature 223:618-619.
Lorén I, Alumets J, Håkanson R, Sundler F (1979) Immuroreactive pancreatic polypeptide (PP) occurs in the central and peripheral nervous system: Preliminary immunocytochemical observations. Cell Tiss Res 200:179--186.
Lorenzi M, Karam JH, Tsalikian E, Bohannon NV, Gerich JE, Forsham PH (1979) Dopamine during α- or β-adrenergic blockade in man. Hormonal, metabolic and cardiovascular effects. J Clin Invest 63:310-317.
Loubatières A, Mariani MM, Sorel G, Savi L (1971) The action of β-adrenergic blocking and stimulating agents on insulin secretion. Characterization of the type of β-receptor. Diabetologia 7:127-132.
Loubatières-Mariani MM, Chapal J, Ribes G, Loubatières AL (1977) Discrepancies in the response of the insulin secreting cells of the dog and rat to different adrenergic stimulating agents. Acta Diabetol Lat 14:144-155.
Luft R, Efendić S, Hökfelt T, Johansson O, Arimura A (1974) Immunohistochemical evidence for the localization of somatostatin-like immunoreactivity in a cell population of the pancreatic islets. Med Biol 52:428-430.
Luft R, Efendić S, Hökfelt T (1978) Somatostatin - Both hormone and neurotransmitter? Diabetologia 14:1-13.
Lundberg JM, Hökfelt T, Nilsson G, Terenius L, Rehfeld J, Elde P, Said S (1978) Peptide neurons in the vagus, splanchnic and sciatic nerves. Acta Physiol Scand 104:499-501.

Lundquist I (1971) Insulin secretion. Its regulation by monoamines and acid amyloglucosidase. Acta Physiol Scand Suppl 372:1-47.
Lundquist I (1972a) Interaction of amines and aminergic blocking agents with blood glucose regulation. I. β-Adrenergic blockade. Europ J Pharmacol 18:213-224.
Lundquist I (1972b) Interaction of amines and aminergic blocking agents with blood glucose regulation. II. α-Adrenergic blockade. Europ J Pharmacol 18:225-235.
Lundquist I, Ericson LE (1978) β-Adrenergic insulin release and adrenergic innervation of mouse pancreatic islets. Cell Tiss Res 193:73-85.
Lundquist I, Ekholm R, Ericson LE (1971) Monoamines in pancreatic islets of the mouse. 5-Hydroxytryptamine as an intracellular modifier of insulin secretion and the hypoglycaemic action of monoamine oxidase inhibitors. Diabetologia 7:414-422.
Lundquist I, Sundler F, Håkanson R, Larsson LI, Heding LG (1975) Differential changes in the 5-hydroxytryptamine and insulin content of guinea-pig B-cells. Endocrinology 97:937-947.
Mahoney C, Feldman JM (1977) Species variation in pancreatic islet monoamine uptake and action. Diabetes 26:257-261.
Malbon CC, Moreno FJ, Cabelli RJ, Fain JN (1978) Fat cell adenylate cyclase and β-adrenergic receptors in altered thyroid states. J Biol Chem 253:671-678.
Marks V (1959) An improved glucose-oxidase method for determining blood, CSF, and urine glucose levels. Clin Chim Acta 4:395-400.
Marliss EB, Girardier L, Seydoux J, Wollheim CB, Kanazawa Y, Orci L, Renold AE, Porte Jr D (1973) Glucagon release induced by pancreatic nerve stimulation in the dog. J Clin Invest 52:1246-1259.
Matthews EK (1977) Insulin secretion. In: Dumont J, Nunez J (eds) Hormones and cell regulation, vol. I, Elsevier/North Holland Biomedical Press, Amsterdam, pp. 57-76.
McCulloch AJ, Nosadini R, Pernet A (1980) Glucose turnover and recycling: Effect of thyroid hormones. Diabetologia 19:298.
McGuigan JE (1968) Gastric mucosal intracellular localization of gastrin by fluorescence. Gastroenterology 55:315-327.
McIntyre N, Holdsworth CD, Turner DS (1964) New interpretation of oral glucose tolerance. Lancet 2:20-21.
Meda P, Amherdt M, Perrelet A, Orci L (1980) Coupling between insulin-containing cells. A morphological approach. In: Waldhäusl WK (ed) Diabetes 1979, Excerpta Medica, Amsterdam Oxford Princeton, pp. 496-501.
Meissner HP (1976) Electrophysiologic evidence for coupling between β-cells of pancreatic islets. Nature 262:502-504.
Minkowski O (1893) Untersuchungen über den Diabetes mellitus nach Extirpation des Pankreas. Naunyn-Schmiedebergs Arch Exp Path Pharmak 31:85-189.
Moody AJ, Sundby F (1980) The structure and biosynthesis of glucagon. In: Waldhäusl WK (ed) Diabetes 1979, Excerpta Medica, Amsterdam Oxford Princeton, pp. 427-431.
Moody AJ, Sundby F, Jacobsen H, Lauritsen KB (1978) The tissue distribution and plasma levels of glicentin (gut GLI-1). Scand J Gastroenterol 13:Suppl 49:127.
Moltz JH, Dobbs RE, McCann SM, Fawcett CP (1977) Effects of hypothalamic factors on insulin and glucagon release from the islets of Langerhans. Endocrinology 101:196-202.
Moore B, Edie ES, Abram JH (1906) On the treatment of diabetes mellitus by acid extract of duodenal mucous membrane. Biochem J 1:28-38.

Mortimer CH, Carr D, Lind T, Bloom SR, Mallinson CN, Schally AV, Tunbridge WMG, Yeomans L, Coy DH, Kastin A, Besser GM, Hall R (1974) Effects of growth-hormone release-inhibiting hormone on circulating glucagon, insulin, and growth hormone in normal, diabetic, acromegalic and hypopituitary patients. Lancet 1:697-701.

Mroz EA, Leeman SE (1977) Substance P. Vitam Horm 35:209-281.

Murlin JR, Clough HG, Gibbs CBF, Stokes AM (1923) Aqueous extracts of pancreas I. Influence on the carbohydrate metabolism of depancreatized animals. J Biol Chem 56:253-296.

Mutt V (1979) Chemistry of cholecystokinins. In: Rehfeld JF, Amdrup E (eds) Gastrins and the vagus. Academic Press, London New York San Francisco, pp 57-71.

Mutt V, Said SI (1974) Structure of the porcine vasoactive intestinal octacosapeptide. The amino-acid sequence. Use of Kallikrein in its determination. Europ J Biochem 42:581-589.

Müller WA, Faloona GR, Unger RH (1971) The effect of experimental insulin deficiency on glucagon secretion. J Clin Invest 50:1992-1999.

Myers MG, Hope-Gill HF (1979) Effect of D- and DL-propranolol on glucose-stimulated insulin release. Clin Pharmacol Therapeut 25:303-308.

Nagai K, Frohman LA (1978) Neurotensin hyperglycemia: Evidence for histamine mediation and the assessment of a possible physiologic role. Diabetes 27:577-582.

Ohneda A, Horigome K, Kai Y, Itabashi H, Ishii S, Yamagata S (1976) Purification of canine gut glucagon-like immunoreactivity (GLI) and its insulin releasing activity. Horm Metab Res 8:170-174.

Ohneda A, Ishii S, Horigome K, Chiba M, Sakai T, Kai Y, Watanabe K, Yamagata S (1977) Effect of intrapancreatic administration of vasoactive intestinal polypeptide upon the release of insulin and glucagon in dogs. Horm Metab Res 9:447-452.

Okajima F, Ui M (1978) Adrenergic modulation of insulin secretion in vivo dependent on thyroid states. Am J Physiol 234:E106-E111.

Oliver JR (1976) Inhibition of calcium uptake by somatostatin in isolated rat islets of Langerhans. Endocrinology 99:910-913.

Ondetti MA, Rubin P, Engel SL, Pluscec J, Sheehan JT (1970) Cholecystokinin-pancreozymin. Recent developments. Am J Digest Dis 15:149-156.

Orci L (1976) A fresh look at the interrelationships within the islets of Langerhans. In: Renold A, Creutzfeldt W, Pfeiffer EF (eds) Diabetes Research Today, FK Schattauer Verlag, Stuttgart New York, pp. 135-152.

Orci L, Stauffacher W, Renold AE, Rouiller C (1970) The ultrastructural aspect of A-cells of nonketotic and ketotic diabetic animals. Indications for stimulation and inhibition of glucagon production. Acta Isotop 10:171-186.

Orci L, Unger RH, Renold AE (1973) Structural coupling between pancreatic islet cells. Experientia 29:1015-1018.

Orci L, Baetens D, Rufener C, Brown M, Vale W, Guillemin R (1976) Evidence for immunoreactive neurotensin in dog intestinal mucosa. Life Sci 19:559-562.

Orunesu M, Fugassa E, Pranzetti P (1969) Liver glycogen metabolism in the hyperthyroid rat. Ital J Biochem 18:327-345.

Östenson CG (1979) Regulation of glucagon release: Effects of insulin on the pancreatic A_2-cell of the guinea pig. Diabetologia 17:325-330.

Pace CS, Murphy M, Conant S, Lacy PE (1977) Somatostatin inhibition of glucose-induced electrical activity in cultured rat islet cells. Am J Physiol 233:C164-C171.

Patzelt C, Chan SJ, Quinn PS, Carroll RJ, Tager HS, Steiner DF (1980) Biosynthetic precursors of glucagon: Identification of proglucagon and pre-proglucagon. In: Waldhäusl WK (ed) Diabetes 1979, Excerpta Medica, Amsterdam Oxford Princeton pp. 119-123.

Pearse AGE, Polak JM (1975) Immunocytochemical localization of substance P in mammalian intestine. Histochemistry 41:373-375.
Pearse AGE, Polak, JM, Bloom SR (1977) The newer gut hormones. Cellular sources, physiology, pathology, and clinical aspects. Gastroenterology 72:746-761.
Pederson RA, Brown JC (1978) Interaction of gastric inhibitory polypeptide, glucose, and arginine on insulin and glucagon secretion from the perfused rat pancreas. Endocrinology 103:610-615.
Pederson RA, Schubert HE, Brown JC (1975) The insulinotropic action of gastric inhibitory polypeptide. Can J Physiol Pharmacol 53:217-223.
Perley MJ, Kipnis DM (1967) Plasma insulin responses to oral and intravenous glucose: Studies in normal and diabetic subjects. J Clin Invest 46:1954-1962.
Polak JM, Bloom SR, Coulling I, Pearse AGE (1971) Immunofluorescent localization of enteroglucagon cells in the gastrointestinal tract of the dog. Gut 12:311-318.
Polak JM, Bloom SR, Kuzio M, Brown JC, Pearse AGE (1973) Cellular localization of gastric inhibitory polypeptide in the duodenum and jejunum. Gut 14:284-288.
Polak JM, Pearse AGE, Garaud JC, Bloom SR (1974) Cellular localization of a vasoactive intestinal peptide in the mammalian and avian gastrointestinal tract. Gut 15:720-724.
Polak JM, Pearse AGE, Bloom SR, Buchan AMJ, Rayford PL, Thompson JC (1975) Identification of cholecystokinin-secreting cells. Lancet 2:1016--1018.
Porte Jr D (1967a) A receptor mechanism for the inhibition of insulin release by epinephrine in man. J Clin Invest 46:86-94.
Porte Jr D (1967b) Beta adrenergic stimulation of insulin release in man. Diabetes 16:150-155.
Porte Jr D, Williams RH (1966) Inhibition of insulin release by norepinephrine in man. Science 152:1248-1250.
Porte Jr D, Girardier L, Seydoux J, Kanazawa Y, Posternak J (1973) Neural regulation of insulin secretion in the dog. J Clin Invest 52:210-214.
Pulido OM, Bencosme SA, de Bold ML, de Bold JA (1978) Intracellular pancreatic B cell serotonin and the dynamics of insulin release. Diabetologia 15:197-204.
Rabinovitch A, Dupré J (1972) Insulinotropic and glucagonotropic activities in crude preparations of cholecystokinin-pancreozymin. Clin Res 20:945.
Rabinovitch A, Dupré J (1974) Effects of the gastric inhibitory polypeptide present in impure pancreozymin-cholecystokinin on plasma insulin and glucagon in the rat. Endocrinology 94:1139-1144.
Rappaport AM, Kawamura T, Davidson JK, Lin BJ, Ohira S, Zeigler M, Coddling JA, Henderson J, Haist RE (1971) Effects of hormones and of blood flow on insulin output of isolated pancreas in situ. Am J Physiol 221:343-348.
Raptis S, Dollinger HC, Schlegel W, Nadjafi AS (1975) Portal and peripheral blood concentrations of insulin and exocrine pancreatic secretion in response to pure (99%) cholecystokinin-pancreozymin (CCK-PZ) in man. Diabetologia 11:371.
Raptis S, Schlegel W, Lehmann E, Dollinger HC, Zoupas Ch (1978) Effects of somatostatin on the exocrine pancreas and the release of duodenal hormones. Metabolism 27:1321-1328.
Ravazzola M, Orci L (1980) Glucagon and glicentin immunoreactivity are topologically segregated in the α granule of the human pancreatic A cell. Nature 284:66-67.

Ravazzola M, Siperstein A, Moody AJ, Sundby F, Jacobsen H, Orci L (1979) Glicentin immunoreactive cells: Their relationship to glucagon-producing cells. Endocrinology 105:499-508.
Rehfeld JF (1971) Effect of gastrin and its C-terminal tetrapeptide on insulin secretion in man. Acta Endocrinol Kbh 66:169-176.
Rehfeld JF (1978) Immunochemical studies on cholecystokinin. II. Distribution and molecular heterogeneity in the central nervous system and small intestine of man and hog. J Biol Chem 253:4022-4030.
Rehfeld JF, Stadil F (1973) The effect of gastrin on basal and glucose--stimulated insulin secretion in man. J Clin Invest 52:1415-1426.
Rehfeld JF, Holst JJ, Kühl C (1978) The effect of gastrin on basal and aminoacid-stimulated insulin and glucagon secretion in man. Europ J Clin Invest 8:5-9.
Rehfeld JF, Larsson LI, Goltermann NR, Schwartz TW, Holst JJ, Jensen SL, Morley JS (1980) Neural regulation of pancreatic hormone secretion by the C-terminal tetrapeptide of CCK. Nature 284:33-38.
Renauld A, Pinto JEB, Sverdlik RC, Foglia VG (1971) Studies on the effect of hyperthyroidism on the insulin response to hyperglycemia in the dog. Horm Metab Res 3:247-251.
Renauld A, Sverdlik RC, Rodriguez RR (1978) Metabolic and endocrine effects of long-term thyroxine treatment. Horm Metab Res 10:568-569.
Rerup C, Lundquist I (1966) Blood glucose level in mice. I. Evaluation of a new technique of multiple serial sampling. Acta Endocrinol Kbh 52:357-367.
Rerup C, Lundquist I (1967) Non-specific reaction of current glucose oxidase preparations with glycogen and its application for glycogen determinations in tissue. Acta Pharmacol Toxicol 25:41-53.
Robertson RP (1979) Prostaglandins as modulators of pancreatic islet function. Diabetes 28:943-948.
Robertson RP, Porte Jr D (1973) Adrenergic modulation of basal insulin secretion in man. Diabetes 22:1-8.
Rubinstein AH, Clark JL, Melani F, Steiner DF (1969) Secretion of proinsulin C-peptide by pancreatic β cells and its circulation in blood. Nature 224:697-699.
Said SI, Mutt V (1970) Polypeptide with broad biological activity: Isolation from small intestine. Science 169:1217-1218.
Samols E, Marri G, Marks V (1965) Promotion of insulin secretion by glucagon. Lancet 2:415-416.
Samols E, Tyler J, Megyesi C, Marks V (1966) Immunochemical glucagon in human pancreas, gut, and plasma. Lancet 2:727-729.
Sasaki H (1976) Effects of substance-P. Metabolism 25:1463.
Schäfer HJ, Klöppel G (1974) The significance of calcium in insulin secretion. Ultrastructural studies on identification and localization of calcium in activated and inactivated B-cells of mice. Virchows Arch A (Pathol Anat) 362:231-245.
Schäfer R, Schatz H (1979) Stimulation of (pro-)insulin biosynthesis and release by gastric inhibitory polypeptide in isolated islets of rat pancreas. Acta Endocrinol Kbh 91:493-500.
Schatz H, Otto J, Hinz M, Maier V, Nierle C, Pfeiffer EF (1974) Gastrointestinal hormones and function of pancreatic islets: Studies on insulin secretion, ^{3}H-leucine incorporation and intracellular free leucine pool in isolated pancreatic mouse islets. Endocrinology 94:248-253.
Schebalin M, Said SI, Makhlouf GM (1977) Stimulation of insulin and glucagon secretion by vasoactive intestinal peptide. Am J Physiol 232:E197-E200.
Sherwin R, Felig P (1978) Pathophysiology of diabetes mellitus. Med Clin N Amer 62:695-711.
Shima K, Kurokawa M, Sawazaki N, Tanaka R, Kumahara (1978) Effect of secretin on plasma insulin and glucagon in man. Endocrinol Jap 25:461-465.

Sive AA, Vinik AI, van Tonder SV (1979) Pancreatic polypeptide (PP) responses to oral and intravenous glucose in man. Am J Gastroenterol 71:183-185.
Smith PH, Woods SC, Porte Jr D (1976) Phentolamine blocks the somatostatin-mediated inhibition of insulin secretion. Endocrinology 98:1073--1076.
Smith PH, Merchant FW, Johnson DG, Fujimoto WY, Williams RH (1977) Immunocytochemical localization of a gastric inhibitory polypeptide-like material within A-cells of the endocrine pancreas. Am J Anat 149:585--590.
Snyder SH, Innis RB (1979) Peptide neurotransmitters. Ann Rev Biochem 48:755-782.
Staehelin LA (1974) Structure and function of intercellular junctions. Int Rev Cytol 39:191-283.
Steffens AB (1980) Neural factors in meal related insulin and glucagon secretion. In: Waldhäusl WK (ed) Diabetes 1979, Excerpta medica, Amsterdam Oxford Princeton. pp. 47-50.
Steiner DF (1969) Proinsulin and the biosynthesis of insulin. New Engl J Med 280:1106-1113.
Sterling K (1979) Thyroid hormone action at the cell level. New Engl J Med 300:117-123 & 173-177.
Sternberger LA (1974) Immunocytochemistry. Prentice-Hall, Eaglewood, Cliffs, New Jersey.
Sundby F, Jacobsen H, Moody AJ (1976) Purification and characterization of a protein from porcine gut with glucagon-like immunoreactivity. Horm Metab Res 8:366-371.
Sundler F, Håkanson R, Hammer RA, Alumets J, Carraway R, Leeman SE, Zimmerman EA (1977a) Immunohistochemical localization of neurotensin in endocrine cells of the gut. Cell Tiss Res 178:313-321.
Sundler, F, Alumets J, Håkanson R, Carraway R, Leeman SE (1977b) Ultrastructure of the gut neurotensin cell. Histochemistry 53:25-34.
Sundler F, Alumets J, Håkanson R, Fahrenkrug J, Schaffalitzky de Muckadell O (1978) Peptidergic (VIP) nerves in the pancreas. Histochemistry 55:173-176.
Sundler F, Håkanson R, Lorén I, Lundquist I (1979) Amine storage and function in peptide hormone-producing cells. Invest Cell Pathol 3:87-103.
Sundler F, Håkanson R, Leander S (1980) Peptidergic nervous systems in the gut. Clin Gastroenterol 9:517-543.
Sutherland EW, de Duve C (1948) Origin and distribution of the hyperglycemic-glycogenolytic factor of the pancreas. J Biol Chem 175:663-674.
Szecówka J, Sandberg E, Efendić S (1980) The interaction of vasoactive intestinal polypeptide (VIP), glucose and arginine on the secretion of insulin, glucagon and somatostatin in the perfused rat pancreas. Diabetologia 19:137-142.
Taborsky Jr GJ, Smith PH, Halter JB, Porte Jr D (1979) Glucose infusion potentiates the acute insulin response to nonglucose stimuli during the infusion of somatostatin. Endocrinology 105:1215-1220.
Taminato T, Seino Y, Goto Y, Inoue Y, Kadowaki S, Mori K, Nozawa M, Yajima H, Imura H (1977) Synthetic gastric inhibitory polypeptide. Stimulatory effect on insulin and glucagon secretion in the rat. Diabetes 26:480-484.
Tanaka R, Matsuyama T, Shima K, Sawazaki N, Tarui S, Kumahara Y (1977) Insulin releasing activity of gastrointestinal glucagon-like immunoreactive materials in perfused rat pancreas. Endocrinol Japon 6:575--579.
Taniguchi H, Utsumi M, Hasegawa M, Kobayashi T, Watanabe Y, Murakami K, Seki M, Tsutou A, Makimura M, Sakoda M, Baba S (1977) Physiologic role of somatostatin. Insulin release from rat islets treated by somatostatin antiserum. Diabetes 26:700-702.

Trimble ER, Siegel EG, Berthoud HR, Renold AE (1980) Intraportal islet transplantation: Functional assessment in conscious unrestrained rats. Endocrinology 106:791-797.
Turner DS, Etheridge L, Marks V, Brown JC, Mutt V (1974) Effectiveness of the intestinal polypeptides, IRP, GIP, VIP and motilin on insulin release in the rat. Diabetologia 10:459-463.
Ukai M, Inoue I, Itatsu T (1977) Effect of somatostatin on neurotensin--induced glucagon release and hyperglycemia. Endocrinology 100:1284--1286.
Unger RH, Orci L (1976) Physiology and pathophysiology of glucagon. Physiol Rev 56:778-826.
Unger RH, Eisentraut AM, McCall MS, Madison LL (1962) Measurements of endogenous glucagon in plasma and the influence of blood glucose concentration upon its secretion. J Clin Invest 41:682-689.
Unger RH, Ketterer H, Dupré J, Eisentraut AM (1967) The effects of secretin, pancreozymin, and gastrin on insulin and glucagon secretion in anesthetized dogs. J Clin Invest 46:630-645.
Unger RH, Ohneda A, Valverde I, Eisentraut AM, Exton J (1968) Characterization of the responses of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. J Clin Invest 47:48-65.
Unger RH, Dobbs RE, Orci L (1978) Insulin, glucagon, and somatostatin secretion in the regulation of metabolism. Ann Rev Physiol 40: 307-343.
Uvnäs-Wallensten K (1980) Luminal secretion of gut peptides. Clin Gastroenterol 9:545-554.
Valverde I, Rigopoulou D, Exton J, Ohneda A, Eisentraut A, Unger RH (1968) Demonstration and characterization of a second fraction of glucagon--like immunoreactivity in jejunal extracts. Am J Med Sci 255:415--420.
Vanderhaeghen JJ, Signeau JC, Gepts W (1975) New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature 257:604-605.
Vizi SE, Bertaccini G, Impicciatore M, Knoll J (1973) Evidence that acetylcholine released by gastrin and related polypeptides contributes to their effect on gastrointestinal motility. Gastroenterology 64: 268-277.
Wahren J, Felig P (1976) Influence of somatostatin on carbohydrate disposal and absorption in diabetes mellitus. Lancet 2:1213-1216.
Wajchenberg BL, Cesar FP, Lema CE, Souza ITT, Pieroni RR, Mattar E (1978) Effects of adrenergic stimulating and blocking agents on glucose--induced insulin responses in human thyrotoxicosis. Metabolism 27: 1715-1720.
Walsh JH, Lam SK (1980) Physiology and pathology of gastrin. Clin Gastroenterol 9:567-592.
Watanabe AM, McConnaughey MM, Strawbridge RA, Flemming JW, Jones LR, Besch Jr HR (1978) Muscarinic cholinergic receptor modulation of β-adrenergic receptor affinity for catecholamines. J Biol Chem 253: 4833-4836.
Westfall TC (1977) Local regulation of adrenergic transmission. Physiol Rev 57:659-728.
Williams JA, Korc M, Dormer RL (1978) Action of secretagogues on a new preparation of functionally intact, isolated pancreatic acini. Am J Physiol 235:E517-E524.
Williams RH, Champagne J (1979) Effects of cholecystokinin, secretin, and pancreatic polypeptide on secretion of gastric inhibitory polypeptide, insulin, and glucagon. Life Sci 25:947-956.
Wisén O, Uvnäs-Wallensten K, Efendić S, Johansson C (1980) Release of gastrin and somatostatin into the gastric lumen of healthy subjects and patients with duodenal ulcer and achlorhydria. Acta Physiol Scand 108:297-300.

Woods SC, Porte Jr D (1974) Neural control of the endocrine pancreas. Physiol Rev 54:596-619.

Wurtman RJ, Chou C, Rose C (1970) The fate of C^{14}-dihydroxyphenylalanine (C^{14}-DOPA) in the whole mouse. J Pharmacol Exptl Ther 174:351-356.

Yamada T, Solomon TE, Petersen H, Levin SR, Lewin K, Walsh JH, Grossman MI (1980) Effects of gastrointestinal polypeptides on hormone content of endocrine pancreas in the rat. Am J Physiol 238:G526-G530.

IX. APPENDIX: Papers 1-13

		Page
Paper 1:	Effects of Two Cholecystokinin Variants, CCK-39 and CCK-8 on Basal and Stimulated Insulin Secretion	71
Paper 2:	Effects of Vasoactive Intestinal Polypeptide (VIP), Secretin and Gastrin on Insulin Secretion in the Mouse	83
Paper 3:	GIP-like Immunoreactivity in Glucagon Cells. Interactions Between GIP and Glucagon on Insulin Release	91
Paper 4:	Effects of Glicentine on Insulin Secretion	105
Paper 5:	Somatostatin, Pancreatic Polypeptide, Substance P, and Neurotensin: Cellular Distribution and Effects on Stimulated Insulin Secretion in the Mouse	111
Paper 6:	Adrenergic Innervation of Pancreatic Islets and Modulation of Insulin Secretion by The Sympatho-Adrenal System	119
Paper 7:	Adrenalectomy and Chemical Sympathectomy: Effects on Basal and Stimulated Insulin Secretion	133
Paper 8:	Effects of Selective and Nonselective β-Adrenergic Agents on Insulin secretion In Vivo	143
Paper 9:	Effects of Autonomic Blockade by Methylatropine and Optical Isomers of Propranolol on Plasma Insulin Levels in the Basal State and after Stimulation	157
Paper 10:	Influence of the Sympatho-Adrenal System and Somatostatin on the Secretion of Insulin in the Rat	167
Paper 11:	Insulin Secretion Induced by Glucose and by Stimulation of β_2-Adrenoceptors in the Rat. Different Sensitivity to Somatostatin	179
Paper 12:	The Role of Intracellular Amines in the Regulation of Islet Cell Function	187
Paper 13:	Insulin Secretory Response to Different Secretagogues in Hyper- and Hypothyroid Mice	195

Effects of Two Cholecystokinin Variants, CCK-39 and CCK-8, on Basal and Stimulated Insulin Secretion

Bo Ahrén and Ingmar Lundquist

Department of Pharmacology, University of Lund, Lund, Sweden

ABSTRACT

The effects of two different cholecystokinin variants, CCK-39 and the carboxyl-terminal octapeptide CCK-8, on basal and stimulated insulin secretion were studied in the mouse. It was found that both peptides stimulated insulin secretion in a dose-dependent manner with a maximal response of similar magnitude at a dose level of about 5 nmol/kg body weight. This dose induced an increase of plasma concentrations of immunoreactive insulin of about 100 μU/ml. In submaximal equimolar doses, CCK-39 was slightly more potent than CCK-8. The insulin secretory response to CCK-39 and CCK-8 in the basal state was partially abolished by pretreatment with the cholinergic blocker methylatropine as well as with the β-adrenergic blocker L-propranolol. Thus, this comparatively great insulin secretory response to the two peptides seemed to be dependent on intact muscarinic and β-adrenergic receptors, respectively. When injecting CCK-39 or CCK-8, in doses substimulatory in the basal state, prior to half-maximal doses of D-glucose, the cholinergic agonist carbachol, or the β-adrenergic agonist L-isopropylnoradrenaline (L-IPNA), respectively, the two peptides displayed different influences on insulin release. CCK-39 potentiated glucose- as well as carbachol-induced insulin secretion, whereas it did not influence L-IPNA-induced insulin release. An equimolar dose of CCK-8, on the contrary, had no apparent effect on either glucose-, carbachol-, or L-IPNA-induced insulin release. This observation indicates that stimulated insulin release is influenced by amino acid sequences other than those of the C-terminal octapeptide in CCK-39 and that the response of the stimulated insulin cells to CCK-39 is dependent on the nature of the secretagogue.

INTRODUCTION

The gastrointestinal hormones are known to influence the secretion of insulin. The great insulin release induced by an oral intake of glucose compared to that obtained by an intravenous administration of the same amount of glucose has been ascribed to be largely mediated by these hormones (see recent rev. 4).

It was early suggested that cholecystokinin (CCK) played a role in this

gastrointestinal potentiation of insulin release (39). CCK is released by intake of a meal (24) and administration of CCK has been shown to stimulate insulin release in several species (13,14,15,20,32,34,36,37,39,41). However, results showing no effect of CCK in this respect have also been published, prescribing the demonstrated insulin secretory effect to be caused mainly by impurities in the CCK preparations (12, 33, cf. 4).

CCK has been shown to occur in several different forms, and CCK variants with 39, 33, and 8 amino acids have been described (6, 27). The physiological significance of these different CCK variants is still far from elucidated although most evidence available suggest that the biological activity of CCK is largely confined to the C-terminal octapeptide (CCK-8) (29).

In the present study the in vivo effects of two different CCK molecules on basal and stimulated insulin release were studied in the mouse. The two forms of CCK used were a pure preparation of CCK-39 (26) and the carboxylterminal octapeptide, CCK-8 (29). A comparison of the effects of these two peptides on basal insulin secretion as well as insulin release stimulated by D-glucose, the cholinergic agonist carbachol, and the ß-adrenergic agonist L-isopropylnoradrenaline (L-IPNA) was performed. Additionally, influences of the muscarinic blocker methylatropine and the ß-adrenergic blocker L-propranolol on CCK-induced insulin secretion were investigated.

METHODS

Animals. Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25-35 g, were used throughout the experiments. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden), and tap water ad libitum before and during the experiments.

Drugs. Pure CCK-39 was kindly donated by Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden. Synthetic CCK-8 (Kinevac®) was from Squibb, New Brunnswick, N.J., USA. Both peptides were dissolved in 0.9% NaCl with addition of 0.1% gelatine to avoid adsorbtion to tubes. D-glucose and carbachol were from British Drug Houses Ltd., Poole, England. L-isopropylnoradrenaline (L-IPNA) was from Sterling-Winthrop Research Institute, Rensselaer, N.Y., USA, L-propranolol from ICI, Macclesfield, England and methylatropine from Vitrum AB, Stockholm, Sweden. They were all dissolved in 0.9% NaCl.

Experiments

Effects of CCK on Basal Insulin Secretion. The peptides were administered intravenously in a tail vein. Ten µl/g body weight was injected rapidly. Several different doses were given. Two minutes after the injection, 250 µl blood was drawn by orbital puncture using commercial constriction pipettes. The mice were unanaesthetized all the time. In initial experiments it was found that the peak level of the concentrations of immunoreactive insulin following an injection of CCK-39 and CCK-8, respectively, was recorded about 2 minutes after the injection.

Effects of Methylatropine and L-Propranolol on CCK-Induced Insulin Release. Methylatropine, 8.2 µmol/kg body weight, L-propranolol, 9.6 µmol/kg body weight, or saline, respectively, was injected intraperitoneally; 10 µl/g body weight was given. Twenty minutes later, CCK-39, 2.12 nmol/kg body weight, or CCK-8, 3.18 nmol/kg body weight, or saline, respectively, was given intravenously; 10 µl/g body weight was given. Control groups injected i.v. with carbachol, 0.16 µmol/kg body weight, or L-IPNA, 0.34 µmol/kg body weight, were included. Two minutes after the i.v. injection, blood

was collected in all groups, except the L-IPNA-injected group, where the blood was sampled after 5.5 minutes.

Effects of CCK on Glucose-, Carbachol-, and L-IPNA-Stimulated Insulin Release. Each of the two peptides was injected intravenously in a tail vein, 0.53 nmol/kg body weight was given; the volume load was 5 µl/g body weight. The dose chosen was without effect on basal insulin secretion. 15 seconds after the peptide injection, a rapid intravenous injection of a half-maximal dose of D-glucose, carbachol, or L-IPNA, respectively, was given. The volume load was, again, 5 µl/g body weight. This sequence of injection was chosen in order to mimic the physiological changes following a meal, where gut and pancreatic peptides may reach the insulin cells before the nutrients (22). Two minutes (D-glucose and carbachol) or 5.5 minutes (L-IPNA), respectively, after the last injection, blood was drawn by the orbital puncture technique. Repeated experiments in this laboratory have shown that maximal concentrations of immunoreactive insulin in mouse plasma following a rapid intravenous injection of D-glucose or carbachol is achieved after about 2 minutes, and following injection of L-IPNA after about 5.5 minutes, respectively. The doses used were 2.78 mmol/kg for D-glucose, 0.16 µmol/kg for carbachol, and 0.34 µmol/kg for L-IPNA, respectively.

Determination of Insulin and Glucose. The concentration of immunoreactive insulin (IRI) in plasma was determined by radioimmunoassay (11), using ^{125}I-labelled pig insulin and guinea pig anti-pig insulin. The plasma glucose concentration was determined enzymatically (1).

Statistics. Means and standard errors of the means are given. Student's t-test was employed for tests of significance.

RESULTS

Effects of CCK on Basal Insulin Secretion

CCK-39 or CCK-8 was injected intravenously to mice in doses ranging from 0.53 nmol/kg to 17 nmol/kg, and plasma concentrations of immunoreactive insulin and glucose were recorded in blood samples taken 2 minutes after the injection. It was found, as can be seen in Fig. 1, that both peptides induced a stimulation of insulin release in a dose-dependent manner. The maximal response was of similar magnitude (about 100 µU/ml) and was achieved at a dose level of about 5 nmol/kg for both peptides. CCK-39 appeared to be slightly more potent in submaximal doses. For CCK-39 as well as for CCK-8, 0.53 nmol/kg was substimulatory.

The mean plasma glucose concentration was 8.6 ± 0.28 mmol/l for all animals in this experimental series. There was no difference between controls and peptide-treated animals.

Effects of Methylatropine and L-Propranolol on CCK-Induced Insulin Release

Methylatropine, L-propranolol, or saline was injected i.p. 20 minutes prior to a rapid i.v. injection of a half-maximal dose of CCK-39, 2.12 nmol/kg, or CCK-8, 3.18 nmol/kg. Two other groups were injected i.v. with half-maximal doses of the cholinergic agonist carbachol, or the ß-adrenergic agonist L-IPNA, after the i.p. injection of methylatropine or L-propranolol, respectively.

Fig. 2 demonstrates the effect of pretreatment with methylatropine on carbachol-, CCK-39-, or CCK-8-induced insulin release. It is seen that methylatropine slightly depressed the basal IRI concentrations, from 24.5 ± 3.8 µU/ml to 14.6 ± 3.0 µU/ml ($p < 0.05$). Furthermore, methylatropine diminished the insulin secretory response to carbachol, CCK-39, and CCK-8,

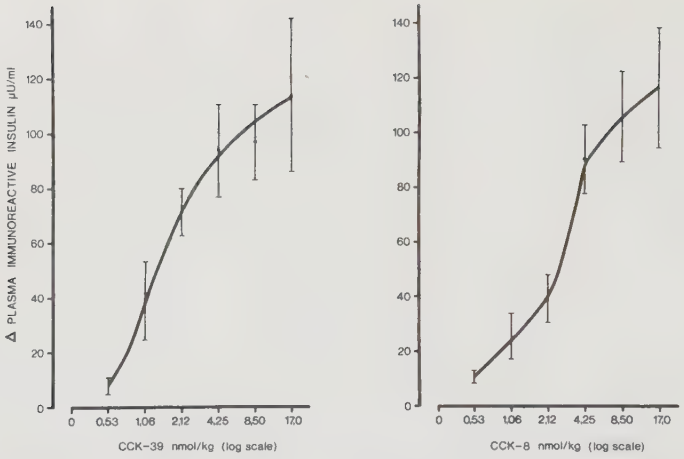


Fig. 1. Dose-response relationships between dose of CCK-39 and CCK-8, respectively, and insulin release. Plasma concentrations of immunoreactive insulin were measured in samples taken 2 minutes after an i.v. injection of the 2 peptides, respectively. Means and SEMs are shown. There were 12 animals in each group. Abscissa: dose of peptide (nmol/kg) (log scale). Ordinate: increase in plasma concentrations of immunoreactive insulin above basal (μU/ml).

respectively. The three secretagogues increased plasma IRI concentrations by about 40-80 μU/ml, and methylatropine depressed this elevation markedly ($p < 0.001$ for all three groups), taking into account the decreased basal plasma insulin levels after methylatropine.

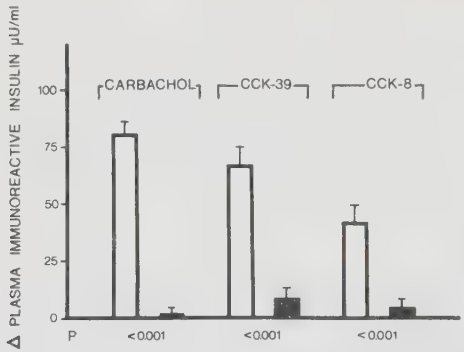


Fig. 2

Increase in plasma concentrations of immunoreactive insulin 2 minutes after the i.v. injection of a half-maximal dose of carbachol (0.16 μmol/kg), CCK-39 (2.12 nmol/kg), or CCK-8 (3.18 nmol/kg). Saline (open columns) or methylatropine (8.2 μmol/kg) (black columns) was injected i.p. 20 minutes before the i.v. injection. There were 22-28 animals in each group. Bars indicate SEMs. P indicates probability level of random difference.

Fig. 3 shows the effect of pretreatment with L-propranolol on L-IPNA-, CCK-39, or CCK-8-induced insulin release. L-propranolol lowered basal plasma IRI concentrations to 13.3 ± 3.6 μU/ml compared to 29.1 ± 3.8 μU/ml ($p < 0.01$) in saline-treated controls. As can be seen in Fig. 3, L-propranolol induced an inhibition of the insulin secretion stimulated by L-IPNA, CCK-39, as well as CCK-8. The three secretagogues elevated the plasma IRI concentrations to a level of about 70-95 μU/ml, and this was depressed to about 20-35 μU/ml by L-propranolol-pretreatment. Thus, the insulin response to L-IPNA ($p < 0.001$), CCK-39 ($p < 0.001$), as well as CCK-8 ($p < 0,01$) was inhibited by L-propranolol, taking into account the depressed basal plasma insulin after β-adrenergic blockade.

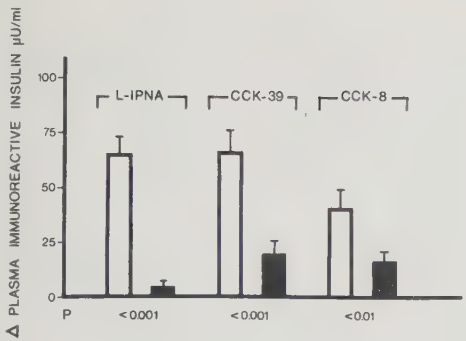


Fig. 3

Increase in plasma concentrations of immunoreactive insulin (peak levels) after the i.v. injection of a half-maximal dose of L-IPNA (0.34 µmol/kg), CCK-39 (2.12 nmol/kg), or CCK-8 (3.18 nmol/kg). Saline (open columns) or L-propranolol (9.6 µmol/kg) (black columns) was injected i.p. 20 minutes before the i.v. injection. There were 22-25 animals in each group. Bars indicate SEMs. P indicates probability level of random difference.

Plasma glucose concentrations were the same for all groups in this series. The control group had a mean value of plasma glucose concentrations of 8.1 ± 0.15 mmol/l. Neither pretreatment with methylatropine or L-propranolol, nor injection of the different secretagogues changed this plasma glucose level during the time-period studied.

Effects of CCK on Glucose-, Carbachol-, and L-IPNA-Stimulated Insulin Release

a) Glucose-Induced Insulin Release. CCK-39, 0.53 nmol/kg, or CCK-8, 0.53 nmol/kg, which is a substimulatory dose on basal insulin release, was injected 15 seconds prior to a half-maximal dose of D-glucose. Control animals were injected with saline and glucose. As can be seen in Fig. 4 the concentration of plasma IRI was increased from 15.7 ± 2.1 µU/ml to 104.7 ± 7.7 µU/ml ($p < 0.001$) following injection of glucose. The substimulatory dose of CCK-39 injected prior to glucose potentiated this glucose-induced insulin response. The plasma IRI concentrations in animals treated with CCK-39 and glucose were 151.6 ± 10.0 µU/ml ($p < 0.005$ vs the glucose-injected control group). Thus, CCK-39 potentiated glucose-induced insulin release by about 45%.

In contrast, an equimolar dose of CCK-8 had no influence on glucose-stimulated insulin secretion. Mean concentration of plasma IRI in animals treated with CCK-8 and glucose was 115.9 ± 9.7 µU/ml (not significantly different from the glucose-injected control group).

The plasma glucose concentrations were increased from 8.4 ± 0.39 mmol/l to 18.3 ± 0.26 mmol/l in response to glucose. There was no difference regarding plasma glucose levels in the three glucose-injected groups.

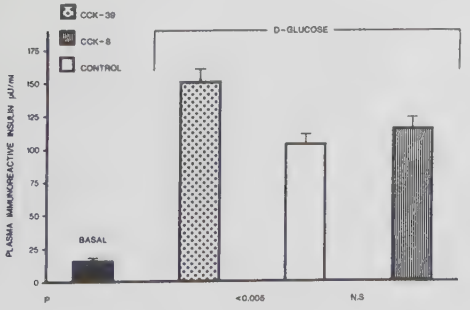


Fig. 4

Plasma concentrations of immunoreactive insulin in the basal state and 2 minutes after the i.v. injection of a half-maximal dose of D-glucose (2.78 mmol/kg). CCK-39 or CCK-8 (0.53 nmol/kg) or in the control group saline was injected i.v. 15 seconds prior to glucose. There were 23 animals in the basal group, and 41-65 animals in each experimental group. Bars indicate SEMs. P indicates probability level of random difference. N.S. not significant.

b) Carbachol-Induced Insulin Release. Each of the two peptides was injected intravenously in the same dose as in the experiments with the glucose-induced insulin release, i.e. 0.53 nmol/kg. Fifteen seconds after this peptide injection, a half-maximal dose of the cholinergic agonist carbachol was injected. Control animals were injected with saline and carbachol.

It was found, as is shown in Fig. 5, that carbachol enhanced the insulin concentration in plasma, from 15.7 $\pm$ 2.1 µU/ml to 116.2 $\pm$ 10.1 µU/ml ($p < 0.001$). CCK-39 potentiated this stimulation of insulin secretion. Plasma immunoreactive insulin concentrations in the group treated with CCK-39 and carbachol were 172.9 $\pm$ 14.3 µU/ml ($p < 0.01$ vs the carbachol- injected control group). CCK-39 thus potentiated carbachol-induced insulin release by about 50%.

The injection of the substimulatory dose of CCK-8 had no influence on carbachol-stimulated secretion of insulin. The plasma insulin level in the CCK-8-carbachol group was 109.3 $\pm$ 9.1 µU/ml (n.s. vs the control). There was no difference in plasma glucose concentrations between the groups.

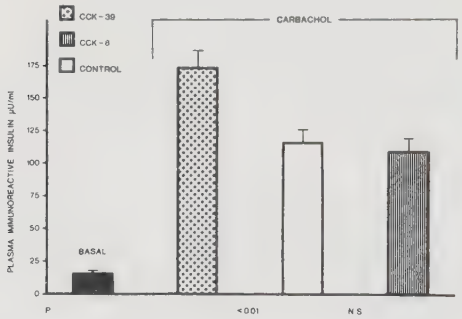


Fig. 5

Plasma concentrations of immunoreactive insulin in the basal state and 2 minutes after the i.v. injection of a half-maximal dose of carbachol (0.16 µmol/kg). CCK-39 or CCK-8 (0.53 nmol/kg) or in the control group saline was injected i.v. 15 seconds prior to carbachol. There were 23 animals in the basal group, and 35-47 animals in each experimental group. Bars indicate SEMs. P indicates probability level of random difference. N.S. not significant.

c) L-IPNA-Induced Insulin Release. The first intravenous injection was the same as in earlier experiments, i.e. 0.53 nmol/kg of CCK-39 or CCK-8. Fifteen seconds following this injection, a half-maximal dose of the β-adrenergic agonist L-IPNA was administered. Control animals were given saline and L-IPNA. In Fig. 6 it can be seen that L-IPNA increased the plasma insulin concentrations, from 9.0 $\pm$ 1.5 µU/ml to 107.5 $\pm$ 10.0 µU/ml ($p < 0.001$). Neither CCK-39 nor CCK-8 displayed any influence on this β-adrenergic stimulation. Plasma immunoreactive insulin concentrations in the CCK-39-L-IPNA group were 113.3 $\pm$ 14.5 µU/ml, and in the CCK-8-L-IPNA group 90.8 $\pm$ 14.2 µU/ml. These values were not different from values obtained in the control group.

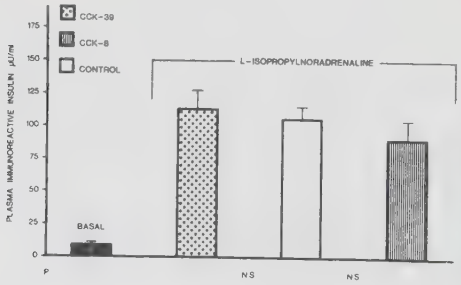


Fig. 6

Plasma concentrations of immunoreactive insulin in the basal state and 5.5 minutes after the i.v. injection of a half-maximal dose of L-IPNA (0.34 µmol/kg). CCK-39 or CCK-8 (0.53 nmol/kg) or in the control group saline was injected i.v. 15 seconds prior to L-IPNA. There were 15-28 animals in each group. Bars indicate SEMs. N.S. not significant.

DISCUSSION

Cholecystokinin is one of the three classically recognized gastrointestinal hormones. It is released by food intake (24) and stimulates secretion of pancreatic enzymes (19, 38) and contraction of smooth muscle in the gastro-intestinal tract (25, 29, 40). CCK has been demonstrated to occur in several different forms in vivo. Variants of CCK with 39, 33, and 8 amino acids, and also in a form which is intermediate between CCK-33 and CCK-8 have been described (6, 27).

CCK cells have been localized to the small intestine, mainly the duodenum, in man and rat (2, 7, 31). A suggestion that the glucagon cells in the pancreatic islets contain CCK (10) has not been supported in other studies (2, 31). Beside in endocrine cells, CCK or CCK-like peptides have been reported to be present also in neural structures (5, 18). CCK may thus have the possibility to influence islet functions both as a hormone and as a neurotransmitter.

Since it was postulated that gastrointestinal hormones played a great role for the insulin release after feeding, studies have been undertaken to clarify the role of CCK in this respect. In man, injection of pure CCK-33-preparations resulted in an increase of portal concentrations of insulin (34), whereas the peripheral concentrations were unchanged (12, 34). CCK-33 also potentiated glucose-induced insulin release (34). Impure preparations of CCK, however, have been shown to induce an increase of also the peripheral concentrations of insulin, but this effect has been ascribed to impurities in the preparations, mainly GIP (gastric inhibitory polypeptide) (12, 32, cf. 4).

In the dog, different CCK molecules, as well as caerulein, which is chemically closely related to CCK-8, stimulate insulin secretion (8,14,15,28, 39,41). Similar results have been found also in the rat, cat, and in the rabbit (13, 20, 30, 33, 37).

In the present study the in vivo effects of two CCK variants on basal and stimulated insulin release were investigated in the mouse. Both CCK-39 and CCK-8 stimulated basal insulin secretion in a dose-dependent manner. The maximal response was the same for the two peptides, but in submaximal doses CCK-39 appeared to be slightly more potent. This is in accordance with results from studies on the force of contraction induced by different CCK molecules on smooth muscle, where CCK-39 and CCK-8 was found to be equipotent (25, 29) but in contrast to reported effects on pancreatic enzyme secretion, where CCK-8 was 10-30 times more potent than CCK-39 (38).

The magnitude of the insulin stimulatory effect of CCK-39 and CCK-8 was found to be decreased by methylatropine as well as by L-propranolol. Both blockers also depressed the basal insulin levels, showing that cholinergic and ß-adrenergic influences are of importance for basal insulin release in the mouse. The importance of the ß-adrenergic influences in this respect has earlier been demonstrated (21). It has been suggested from studies on guinea-pig ileum that CCK-8 might exert its effects through releasing the cholinergic neurotransmitter from nerve terminals (40). Such a mechanism may be compatible with the results from the present study since cholinergic fibers have been described in the islets (3) and since methylatropine inhibited the CCK-effect. However, it does not explain the observation that blockade of the ß-adrenergic receptors as well inhibited CCK--induced insulin release. Thus, CCK might exert its effect on the insulin cell through mechanisms which are related to the muscarinic as well as to the ß-adrenergic receptors. This must not imply that the peptides directly interfere with these receptors, but instead suggests closely connected insulin secretory mechanisms after stimulation by CCK and the secretory events following stimulation of the muscarinic and ß-adrenergic receptors.

CCK and the closely related peptide caerulein have been shown to stimulate outflow of calcium from and activity of adenylate cyclase in pancreatic acinar cells (35, 38). In order to explain these events at least two kinds of CCK-sensitive receptors have been suggested to exist, one type mediating the CCK-effect on calcium fluxes, other types mediating the CCK-effect on adenylate cyclase (35). Further, it has been argued that the ß-adrenergic receptors are more closely related to the adenylate cyclase-cAMP-system (17), whereas the muscarinic receptors are more dependent on ionic changes (9). Thus, if also the insulin secretory response to CCK in the basal state is linked to these two effector systems, blocking of either the muscarinic or the ß-adrenergic receptors might reduce CCK-induced insulin release, as was observed in the present study.

While the effects of CCK-39 and CCK-8 on basal insulin secretion were similar, their effects on stimulated insulin secretion differed. CCK-39 potentiated glucose- as well as carbachol-induced insulin release, while it had no effect on L-IPNA-stimulated insulin secretion. A similar dose of CCK-8, on the other hand, did not influence the insulin secretory response to any of these secretagogues. Thus, CCK-39 had, beside a potent insulin stimulatory activity in the basal state, also the capability to enhance the insulin secretory response to glucose and carbachol, respectively. Since an equimolar dose of CCK-8 did not show this potentiating effect other amino acids than those of the C-terminal octapeptide of CCK-39 appear to be necessary to express full biological activity in this respect.

The potentiation of the insulin releasing effects of glucose and carbachol induced by CCK-39 might be exerted through an increased affinity for effectors of these two stimulators (cf. 16). Since the effects of both glucose and cholinergic agonists seem to be calcium-dependent (9, 23), it might be speculated that the potentiating effect of CCK-39 on these secretagogues is exerted mainly via stimulation of events linked to calcium movements.

In conclusion, CCK-39 and CCK-8 both stimulate insulin secretion in a dose-dependent manner in the mouse. This secretory response to the peptides is inhibited by blocking of either the muscarinic or the ß-adrenergic receptors. Furthermore, CCK-39 potentiates insulin secretion induced by glucose and by the cholinergic agonist carbachol, whereas it was without effect on insulin secretion stimulated by the ß-adrenergic agonist L-IPNA. An equimolar dose of CCK-8 had no effect on stimulated insulin release.

It is suggested that the comparatively great insulin secretory capacity displayed by CCK-39 and CCK-8 is exerted by stimulating insulin secretory pathways which partially are related to the muscarinic and ß-adrenergic receptors, respectively. For the potentiating effect of CCK-39 on glucose- and carbachol-induced insulin release other amino acid sequences than those of the C-terminal octapeptide appear to be of importance.

ACKNOWLEDGEMENTS

The authors express their appreciation to Åsa Amilon, Lena Kvist and Peter Okmark for technical assistance and to Eva Björkbom for secretarial help.

This study was supported by the Swedish Medical Research Council (04P--4289, 14X-4289) and by grants from the Medical Faculty, University of Lund, Lund, Sweden.

REFERENCES

1. BRUSS, M.L., AND A.L. BLACK. Enzymatic microdetermination of glycogen. Anal. Biochem 84:309-312, 1978.
2. BUFFA, R., E. SOLCIÀ, AND V.L.W. GO. Immunohistochemical identification of the cholecystokinin cell in the intestinal mucosa. Gastroenterology 70:528-532, 1976.
3. COUPLAND, R.E. The innervation of the pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. J. Anat. 92:143-149, 1958.
4. CREUTZFELDT, W. The incretin concept today. Diabetologia 16:75-85, 1979.
5. DOCKRAY, G.J. Immunochemical evidence of cholecystokinin-like peptides in brain. Nature 264:568-570, 1976.
6. DOCKRAY, G.J. Immunochemistry of gastrin and cholecystokinin: Development and application of region specific antisera. In: REHFELD, J.F., AND E. AMDRUP. Gastrins and the vagus. Academic Press, London, New York, San Francisco, pp. 73-83, 1979.
7. DUBOIS, P.M., C. PAULIN, AND J.A. CHAYVIALLE. Identification of gastrin-secreting cells and cholecystokinin-secreting cells in the gastrointestinal tract of the human fetus and adult man. Cell Tissue Res. 175:351-356, 1976.
8. FRAME, C.M., M.B. DAVIDSON, AND R.A.L. STURDEVANT. Effects of the octapeptide of cholecystokinin on insulin and glucagon secretion in the dog. Endocrinology 97:549-553, 1975.
9. GAGERMAN, E., L.-Å. IDAHL, H.P. MEISSNER, AND I.-B. TÄLJEDAL. Insulin release, cGMP, cAMP, and membrane potential in acetylcholine-stimulated islets. Am. J. Physiol. 235:E 493-E 500, 1978.
10. GRUBE, D., V. MAIER, S. RAPTIS, AND W. SCHLEGEL. Immunoreactivity of the endocrine pancreas. Evidence for the presence of cholecystokinin-pancreozymin within the A-cell. Histochemistry 56:13-35, 1978.
11. HEDING, L. A simplified insulin radioimmunoassay method. In: DONATO, L., G. MILHAUD, AND J. SIRCHIS. Labelled proteins in tracer studies. Euratom, Brussels pp. 345-350, 1966.
12. HEDNER, P., G. PERSSON, AND D. URSING. Insulin release in fasting man induced by impure but not by pure preparations of cholecystokinin. Acta Med. Scand. 197:109-112, 1975.
13. HINZ, M., N. KATSILAMBROS, B. SCHWEITZER, S. RAPTIS, AND E.F. PFEIFFER. The role of the exocrine pancreas in the stimulation of insulin secretion by intestinal hormones. Diabetologia 7:1-5, 1971.
14. IPP, E., R.E. DOBBS, A. ARIMURA, W. VALE, V. HARRIS, AND R.H. UNGER. Release of immunoreactive somatostatin from the pancreas in response to glucose, amino acids, pancreozymin-cholecystokinin, and tolbutamide. J. Clin. Invest. 60:760-765, 1977.
15. IPP, E., R.E. DOBBS, V. HARRIS, A. ARIMURA, W. VALE AND R.M. UNGER. The effects of gastrin, gastric inhibitory polypeptide, secretin, and the octapeptide of cholecystokinin upon immunoreactive somatostatin release by the perfused canine pancreas. J. Clin. Invest. 60:1216-1219, 1977.
16. JACOBS, S., AND P. CUATRECASAS. The mobile receptor hypothesis and "cooperativity" of hormone binding. Application to insulin. Biochim. Biophys. Acta 433:482-495, 1976.
17. KUO, W.N., D.S. HODGINS, AND J.F. KUO. Adenylate cyclase in islets of Langerhans. Isolation of islets and regulation of adenylate cyclase activity by various hormones and agents. J. Biol. Chem. 248:2705-2711, 1973.
18. LARSSON, L.-I., AND J.F. REHFELD. Peptidergic and adrenergic innervation of pancreatic ganglia. Scand. J. Gastroent. 14:433-437, 1979.

19. LONG, B.W., AND J.D. GARDNER. Effects of cholecystokinin on adenylate cyclase activity in dispersed pancreatic acinar cells. Gastroenterology 73:1008-1014, 1977.
20. LUCKE, C., B. HÖFFKEN, M.J. MITZKAT, AND I. TRAUTSCHOLD. Effect of 2-deoxy-D-glucose and mannoheptulose on the insulin response to pancreozymin in rabbits. Horm. Metab. Res. 6:21-25, 1974.
21. LUNDQUIST, I. Interaction of amines and aminergic blocking agents with blood glucose regulation. I. ß-Adrenergic blockade. Europ. J. Pharmacol. 18:213-224, 1972.
22. LUNDQUIST, I., F. SUNDLER, B. AHREN, J. ALUMETS, AND R. HÅKANSON. Somatostatin, pancreatic polypeptide, substance P, and neurotensin: Cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology 104:832-838, 1979.
23. MALAISSE, W.J., G.R. BRISSON, AND L.E. BAIRD. Stimulus-secretion coupling of glucose-induced insulin release. X. Effect of glucose on ^{45}Ca efflux from perifused islets. Am. J. Physiol. 224:389-394, 1973.
24. MARSHALL, C.E., E.H. EGBERTS, AND A.G. JOHNSON. An improved method for estimating cholecystokinin in human serum. J. Endocrinol. 79:17-27, 1978.
25. MORGAN, K.G., P.F. SCHMALZ, V.L.W. GO, AND J.H. SZURSZEWSKI. Electrical and mechanical effects of molecular variants of CCK on antral smooth muscle. Am. J. Physiol. 235:E 324-E 329, 1978.
26. MUTT, V. Further investigations on intestinal hormonal polypeptides. Clin. Endocrinol. 5 (suppl):175s-183s, 1976.
27. MUTT, V. Chemistry of the cholecystokinins. In: REHFELD, J.F., AND E. AMDRUP. Gastrins and the vagus. Academic Press, London, New York, San Francisco, pp. 57-71, 1979.
28. OHNEDA, A., K. HORIGOME, S. ISHII, Y. KAI, AND M. CHIBA. Effect of caerulein upon insulin and glucagon secretion in dogs. Horm. Metab. Res. 10:7-11, 1978.
29. ONDETTI, M.A., B. RUBIN, S. ENGEL, J. PLUSCEC, AND J.T. SHEEHAN. Cholecystokinin-pancreozymin. Recent developments. Am. J. Digest. Diseases 15:149-156, 1970.
30. OTSUKI, M., C. SAKAMOTO, M. MAEDA, H. YUV, S. MORITA, AND S. BABA. Effect of caerulein on exocrine and endocrine pancreas in the rat. Endocrinology 105:1396-1399, 1979.
31. POLAK, J.M., A.G.E. PEARSE, S.R. BLOOM, A.M.J. BUCHAN, P.L. RAYFORD, AND J.C. THOMPSON. Identification of cholecystokinin-secreting cells. Lancet II:1016-1018, 1975.
32. RABINOVITCH, A., AND J. DUPRE. Insulinotropic and glucagonotropic activities in crude preparations of cholecystokinin-pancreozymin. Clin. Res. 20:945, 1972.
33. RABINOVITCH, A., AND J. DUPRE. Effects of the gastric inhibitory polypeptide present in impure pancreozymin-cholecystokinin on plasma insulin and glucagon in the rat. Endocrinology 94:1139-1144, 1974.
34. RAPTIS, S., H.C. DOLLINGER, W. SCHLEGEL, AND A.S. NADJAFI. Portal and peripheral blood concentrations of insulin and exocrine pancreatic secretion in response to pure (99%) cholecystokinin-pancreozymin (CCK) in man. Diabetologia 11:371, 1975.
35. ROBBERECHT, P., M. DESHODT-LANCKMAN, J.-L. MORGAT, AND J. CHRISTOPHE. The interaction of caerulein with the rat pancreas. 3. Structural requirements for in vitro binding of caerulein-like peptides and its relationship to increased calcium outflux, adenylate cyclase activation, and secretion. Eur. J. Biochem. 91:39-48, 1978.
36. SCHATZ, M., J. OTTO, M. HINZ, V. MAIER, C. NIERLE, AND E.F. PFEIFFER. Gastrointestinal hormones and function of pancreatic islets: Studies on insulin secretion, ^{3}H-leucine incorporation and intracellular free leucine pool in isolated pancreatic mouse islets. Endocrinology 94:248-253, 1974.

37. SCHEBALIN, M., S.I. SAID, AND G.M. MAKHLOUF. Stimulation of insulin and glucagon secretion by vasoactive intestinal peptide. Am. J. Physiol. 232:E197 - E200, 1977.
38. SJODIN, L., AND J.D. GARDNER. Effect of cholecystokinin variant (CCK--39) on dispersed acinar cells from guinea pig pancreas. Gastroenterology 73:1015-1018, 1977.
39. UNGER, R.H., H. KETTERER, J. DUPRE, AND A.M. EISENTRAUT. The effects of secretin, pancreozymin, and gastrin on insulin and glucagon secretion in anesthetized dogs. J. Clin. Invest. 46:630-645, 1967.
40. VIZI, S.E., G. BERTACCINI, M. IMPICCIATORE, AND J. KNOLL. Evidence that acetylcholine released by gastrin and related polypeptides contributes to their effect on gastrointestinal motility. Gastroenterology 64:268-277, 1973.
41. WILLIAMS, R.M., AND J. CHAMPAGNE. Effects of cholecystokinin, secretin, and pancreatic polypeptide on secretion of gastric inhibitory polypeptide, insulin, and glucagon. Life Sci. 25:947-956, 1979.

Effects of Vasoactive Intestinal Polypeptide (VIP), Secretin and Gastrin on Insulin Secretion in the Mouse

Bo Ahrén and Ingmar Lundquist

Department of Pharmacology, University of Lund, Lund, Sweden

Summary. The in vivo effects of vasoactive intestinal polypeptide (VIP), secretin and two different molecular forms of gastrin, gastrin 17 and pentagastrin, on basal and stimulated insulin secretion have been investigated in the mouse. All these peptides induced a moderate dose-dependent increase in basal insulin secretion. The different polypeptides showed complex effects on insulin release stimulated by glucose, the cholinergic agonist carbachol or the β-adrenergic agonist L-isopropylnoradrenaline (L-IPNA), these effects being dependent on the nature of the secretagogue. VIP and secretin both potentiated glucose-induced insulin release. Secretin inhibited insulin secretion induced by carbachol and L-IPNA, whereas VIP potentiated L-IPNA-induced insulin secretion and had no influence on the effect of carbachol. Gastrin 17 and pentagastrin did not affect glucose- or carbachol-induced insulin release, whereas they inhibited L-IPNA-induced insulin secretion. The results suggest that VIP, secretin and gastrin display their effects on insulin secretion through different mechanisms. The results indirectly suggest the existence of separate insulin secretory pathways which operate differently, or at least partly differently, after glucose stimulation, cholinergic stimulation, and β-adrenergic stimulation.

INTRODUCTION

Several gastrointestinal endocrine polypeptides are known to affect insulin secretion (1, 2). Oral intake of glucose is followed by a greater insulin release than IV glucose administration, despite a lower blood glucose level. This potentiated response seems to be due to the release of gastro-intestinal peptides, the so-called incretin effect (3). Peptides produced by intrapancreatic cells, endocrine and neural, may also regulate insulin secretion by a paracrine effect (4). Some of these polypeptides show similarities in chemical structure and can therefore be grouped into two families (5), the glucagon family including glucagon, vasoactive intestinal polypeptide (VIP), secretin, and gastric inhibitory polypeptide (GIP), and the gastrin family including cholecystokinin (CCK) and gastrin.

Recently it was demonstrated that some of the newly discovered gastro-

intestinal poplypeptides interfere with the insulin secretory mechanisms in a complex manner, the effects on stimulated insulin secretion being dependent on the nature of the secretagogue (6). The aim of the present investigation was to characterise further the effect of gastrointestinal polypeptides on basal and stimulated insulin release, and to compare effects of peptides belonging to the same polypeptide family with each other and with members of the other family. For this purpose we selected VIP and secretion on one hand and two different molecular forms of gastrin on the other. The in vivo insulin-releasing properties of these polypeptides were investigated under identical experimental conditions in the mouse.

MATERIALS AND METHODS

Animals.

Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25-35 g, were used. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum before and throughout the experiments.

Drugs.

Pure porcine VIP and pure porcine secretin were kindly donated by Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden. Pentagastrin (Peptavlon^{R}) was obtained from ICI Ltd., Macclesfield, England. Synthetic human gastrin 17 was from Bachem Inc., Torrance, Calif., USA. All peptides were dissolved in 0.154 mol/l saline with the addition of 1 g/l gelatine to avoid adsorption.

D-glucose and carbachol (British Drug Houses Ltd., Poole, England) and L-isopropylnoradrenaline (L-IPNA) (Hässle AB, Mölndal, Sweden) were dissolved in 0.154 mol/l saline.

Protocols

I. Effects on Basal Insulin Secretion. The peptides were administered IV through a tail vein, in a volume of 10 µl/g body weight. Control animals were injected with 0.154 mol/l saline. After 2 or 5.5 min 250 µl blood was drawn by orbital puncture (7). The mice were conscious throughout the experiments.

II. Effects on Stimulated Insulin Secretion. The peptides were injected IV, 5 µl/g, 15 sec prior to a rapid IV injection of 5 µl/g of either D--glucose, carbachol or L-IPNA. Control animals were injected with 0.154 mol/l saline and one of the secretagogues, or with saline alone. This sequence of the injections was chosen in order to mimic the anticipated humoral insulin releasing influence, when gut peptides released following a meal may reach the islet cells before the nutrients (6). Two min (glucose and carbachol) or 5.5 min (L-IPNA) after the last injection blood was collected as above. Previous experiments have shown that the maximal concentration of immunoreactive insulin in mouse plasma following a rapid IV injection of these secretagogues was achieved after 2 (glucose and carbachol) and 5.5 (L-IPNA) min, respectively. The secretagogues were administed in doses giving half-maximal insulin secretory responses. These doses were 2.8 mmol/kg (glucose), 160 nmol/kg (carbachol) and 340 nmol/kg (L-IPNA).

Determination of insulin and glucose

Plasma immunoreactive insulin concentrations (IRI) were measured by radioimmunoassay (8) using ^{125}I-labelled porcine insulin and guinea pig anti-porcine insulin, with porcine insulin as standard. Plasma glucose concentrations were measured with a glucose oxidase method (9).

Statistics

The results are presented as mean ± SEM. Student's t-test was used to test significance.

RESULTS

I. Effects of the Polypeptides on Basal Insulin Secretion

VIP, secretin, gastrin 17 and pentagastrin all induced stimulation of insulin secretion in a dose-dependent manner 2 min after injection (Fig. 1). At the highest dose levels studied they increased the plasma IRI concentrations by 8-30 µU/ml. Plasma IRI did not differ from control values 5.5 min after hormone injection.

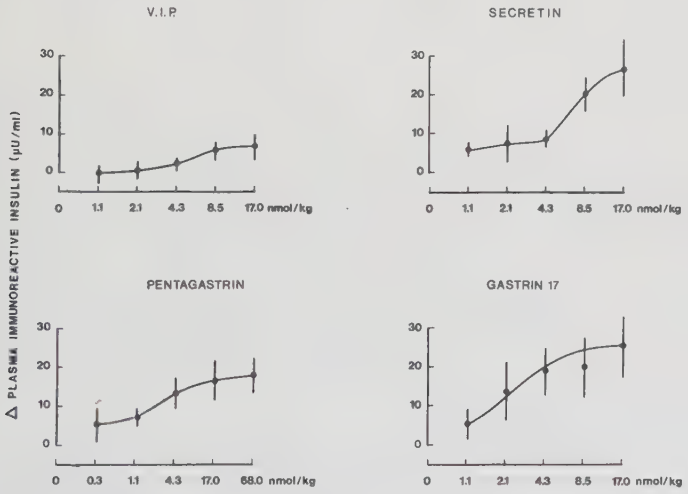


Fig. 1. Dose-response relationships between dose of vasoactive intestinal polypeptide (VIP), secretin, pentagastrin, and gastrin 17, and insulin release in NMRI mice. Plasma concentrations of immunoreactive insulin were measured in samples taken 2 min after IV injection of the polypeptides. Mean ± SEM is shown. Each group consisted of 10-16 animals with the exception of the VIP-experiments when 18-30 mice were used.

II. Effects of the Polypeptides on Stimulated Insulin Secretion

a) VIP (Fig. 2). Injection of VIP, 4.25 nmol/kg, (the threshold dose for stimulation of basal insulin secretion), IV 15 s prior to IV injection of one of the secretagogues induced a potentiation of both glucose- and L-IPNA-stimulated insulin secretion, while carbachol-induced insulin release was unaffected. Glucose-stimulated insulin secretion was increased by 30% ($p < 0.05$), and L-IPNA-induced insulin release by 65% ($p < 0.01$), by VIP.

b) Secretin (Fig. 3). IV injection of secretin, 4.25 nmol/kg, 15 s prior

to IV injection of glucose resulted in a potentiation of glucose-induced insulin release. Secretin was more potent than VIP, potentiating the insulin releasing effect of glucose by 90% ($p < 0.001$). In contrast to VIP, secretin inhibited the effects of both carbachol and L-IPNA. Carbachol-induced insulin secretion was inhibited by 35% ($p < 0.01$) and L-IPNA-stimulated insulin release by 30% ($p < 0.05$).

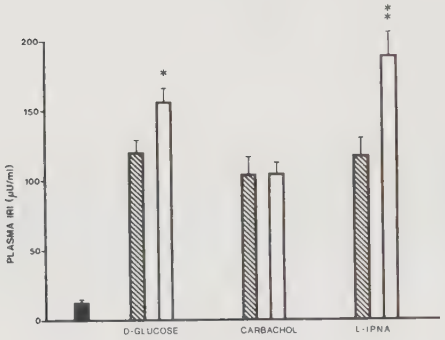


Fig. 2

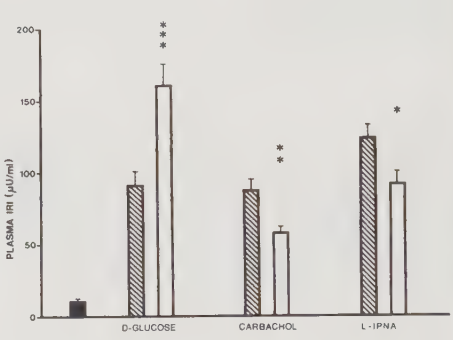


Fig. 3

Figs. 2 and 3. Effect of vasoactive intestinal polypeptide (VIP) (Fig. 2) or secretin (Fig. 3) on acute insulin release stimulated by D-glucose, carbachol, or L-isopropylnoradrenaline (L-IPNA). Saline (0.154 mol/l) (hatched columns) or each of the polypeptides (4.25 nmol/kg) (open columns) was injected IV 15 s prior to half-maximal doses of D-glucose (2.8 mmol/kg), carbachol (160 nmol/kg), or L-IPNA (340 nmol/kg). Control animals received saline (0.154 mol/l) + saline (0.154 mol/l) (black column). Bars indicate SEM. Asterisks indicate probability level of random difference for saline vs polypeptide treated group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Each group consisted of 20-35 mice (Fig. 2) and of 22-45 mice (Fig. 3).

c) Gastrin 17 (Fig. 4) and Pentagastrin (Fig. 5). These peptides exerted identical effects on stimulated insulin release. With IV injection in a dose of 4.25 nmol/kg 15 seconds prior to injection of each of the secretagogues neither of them changed the insulin secretory response to glucose or carbachol. However, both peptides inhibited the L-IPNA-induced insulin secretion, gastrin 17 by 35% ($p < 0.05$) and pentagastrin by 20% ($p < 0.05$).

III. Plasma Glucose Concentrations

Plasma glucose concentrations were 8.5 ± 0.4 mmol/l in mice not receiving glucose. There was no difference in plasma glucose levels between the groups. Thus none of the polypeptides, nor carbachol and L-IPNA influenced the plasma glucose during the time-period studied. Two minutes after glucose injection the plasma glucose concentrations were 15.8 ± 1.1 mmol/l. There was no difference between the glucose-treated groups.

DISCUSSION

The 28 amino acid peptide VIP is presumed to occur in neurones in the gut and pancreas and it has been suggested to be a neurotransmitter (10-13). The 27 amino acid peptide secretin has been shown to occur in endocrine cells in the small intestine (14). Chemically VIP and secretin resemble glucagon and GIP, and these polypeptides have been grouped in one family (5).

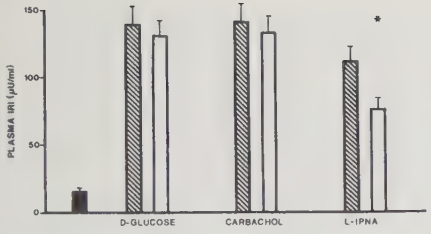


Fig. 4

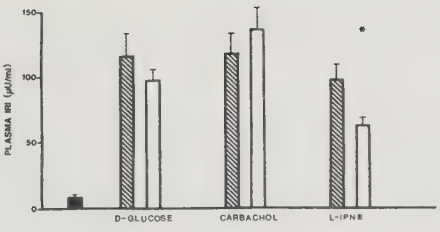


Fig. 5

Figs. 4 and 5. Effects of gastrin 17 (Fig. 4) or pentagastrin (Fig. 5) on acute insulin release stimulated by D-glucose, carbachol, or L-isopropylnoradrenaline (L-IPNA). Saline (0.154 mol/l) (hatched columns) or each of the polypeptides (4.25 nmol/kg) (open columns) was injected IV 15 s prior to half-maximal doses of D-glucose (2.8 mmol/kg), carbachol (160 nmol/kg), or L-IPNA (340 nmol/kg). Control animals received saline (0.154 mol/l) + saline (0.154 mol/l) (black column). Bars indicate SEM. Asterisk indicates probability level of random difference for saline vs polypeptide treated groups. * $p < 0.05$. Each group consisted of 15-33 mice (Fig. 4) and of 10-19 mice (Fig. 5).

VIP has been shown to have a stimulatory action on insulin release in vivo in the dog and pig (15-18) and in the perfused cat pancreas (19), but is reported to have no insulin releasing activity in vivo in the rat (20). Secretin seems to stimulate insulin secretion in high doses in man (1, 2, 21-23) and while augmenting glucose-induced insulin release it had no influence on the effect of other secretagogues (22). In doses that reproduce the level of circulating secretin, however, there was no effect on glucose-induced insulin release (24).

In the present study we have demonstrated a slight dose-dependent increase of basal plasma insulin levels after administration of VIP or secretin and these two peptides also potentiated glucose-induced insulin secretion. It is notable that the insulin-releasing activity of the two polypeptides was very weak compared to the effect of glucose and it seems unlikely that they function as important modulators of basal insulin release. In equimolar doses we found secretin to be more potent than VIP in stimulating basal insulin secretion, and also in its ability to potentiate glucose-induced insulin secretion.

VIP and secretin differed in their effects on carbachol- and L-IPNA-induced insulin release. While VIP enhanced the response to ß-adrenergic stimulation it had no effect on cholinergically induced insulin secretion. Secretin, on the contrary, inhibited the effects of these two secretagogues. It is thus reasonable to assume that in the mouse VIP and secretin interact with different receptors on the insulin cell. From studies in liver and fat cells it was suggested that the two peptides stimulate the same receptors (25), whereas on pancreatic acinar cell membranes from the guinea-pig two kinds of VIP/secretin-sensitive receptors have been described (26). These receptors had different affinities but the same expression for VIP and secretin, respectively. Since the present study demonstrates that some of the effects of VIP and secretin differed it can be suggested that the peptides stimulated less related receptors on the insulin cells.

Gastrin is chemically heterogenous and occurs in several different molecular forms (27). It has been localized to endocrine cells in the antrum and proximal duodenum (28-29). Furthermore it has been demonstrated to

occur in endocrine cells in the pancreatic islets during the neonatal period (30). Recently, gastrin-immunoreactivity was also described in nerves in several organs including the pancreas (13, 31).

A stimulatory effect on basal as well as on glucose-induced insulin release after administration of gastrin to man and dog has previously been reported (1, 2, 21, 32-34). Generally, higher plasma levels of gastrin than are seen under physiological conditions are necessary to obtain an insulin-releasing effect (1). We find that both gastrin 17 and pentagastrin stimulate basal insulin release in a dose-dependent manner, but their potency was low. The results suggest that circulating gastrin is of no or little importance for basal insulin release in the mouse.

Gastrin 17 and pentagastrin were found to exert identical effects on stimulated insulin release, suggesting that they interact with the same receptors. Both peptides inhibited L-IPNA-induced insulin release, while showing no influence on glucose- or carbachol-stimulated secretion of insulin. This pattern of effects differed from that of VIP and secretin. It has been suggested that secretin and gastrin act on the same receptors (35) but since the effects on insulin release of gastrin clearly differed from that of secretin, the results of the present study do not favour this hypothesis.

The physiological relevance of these polypeptides for insulin secretion cannot be deduced from this study. Our results show, however, that insulin secretion is influenced by the gastrointestinal polypeptides in a complex way. Interaction of all polypeptides with one type of receptors on the insulin cell cannot explain the results. Furthermore, the effects of the polypeptides on stimulated insulin secretion are highly dependent on the nature of the secretagogue. The results indirectly suggest that glucose, cholinergic agonists, and ß-adrenergic agonists, respectively, stimulate insulin release by activation of separate or partly separate pathways. The polypeptides could be affecting the receptors of the secretagogues, or initiating intracellular events interacting with those of the secretagogues.

ACKNOWLEDGEMENTS

The technical assistance of Åsa Amilon, Lena Kvist and Peter Okmark is gratefully acknowledged. This study was supported by grants from the Swedish Medical Research Council and from the Medical Faculty, University of Lund, Lund, Sweden.

REFERENCES

1. Rehfeld, J.F.: Gastrointestinal hormones and insulin secretion. Scand. J. Gastroent. 7:289-292 (1972)
2. Brown, J.C., Otte, S.C.: Gastrointestinal hormones and the control of insulin secretion. Diabetes 27:782-787 (1978)
3. Creutzfeldt, W.: The incretin concept today. Diabetologia 16:75-85 (1979).
4. Unger, R.H., Orci, L.: Possible roles of the pancreatic D-cell in the normal and diabetic states. Diabetes 26:241-244 (1977).
5. Dockray, G.J.: Molecular evolution of gut hormones: Application of comparative studies on the regulation of digestion. Gastroenterology 72:344-358 (1977)

6. Lundquist, I., Sundler, F., Ahrén, B., Alumets, J., Håkanson, R.: Somatostatin, pancreatic polypeptide, substance P, and neurotensin: Cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology 104:832-838 (1979).
7. Rerup, C., Lundquist, I.: Blood glucose level in mice. I. Evaluation of a new technique of multiple serial sampling. Acta Endocrinol. (Kbh) 52:357-367 (1966)
8. Heding, L.: A simplified insulin radioimmunoassay method. In: Donato, L., Milhaud, G., Sirchis, J. (eds.): Labelled proteins in tracer studies. Euratom, Brussels, 1966, pp. 345-350
9. Bruss, M.L., Black, A.L.: Enzymatic microdetermination of glycogen. Anal. Biochem. 84:309-312 (1978)
10. Larsson, L.-I., Fahrenkrug. J., Schaffalitzky de Muckadell, O., Sundler, F., Håkanson, R., Rehfeld, J.F.: Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral neurons. Proc. Natl. Acad. Sci. (USA) 73:3197-3200 (1976)
11. Sundler, F., Alumets, J., Håkanson, R., Fahrenkrug, J., Schaffalitzky de Muckadell, O.: Peptidergic (VIP) nerves in pancreas. Histochemistry 55:173-176 (1978)
12. Fahrenkrug, J.: Vasoactive intestinal polypeptide: Measurement, distribution and putative neurotransmitter function. Digestion 19: 149-169 (1979)
13. Larsson, L.-I., Rehfeld, J.F.: Peptidergic and adrenergic innervation of pancreatic ganglia. Scand. J. Gastroent. 14:433-437 (1979)
14. Larsson, L.-I., Sundler, F., Alumets, J., Håkanson, R., Schaffalitzky de Muckadell, O.B., Fahrenkrug, J.: Distribution, ontogeny and ultrastructure of the mammalian secretin cell. Cell Tiss. Res. 181:361-368 (1977)
15. Lindkear Jensen, S., Fahrenkrug, J., Holst, J.J., Vagn Nielsen, O., Schaffalitzky de Muckadell, O.B.: Secretory effects of vasoactive intestinal polypeptide (VIP) on the isolated perfused porcine pancreas. Am. J. Physiol. 235:E 387-E 391 (1978)
16. Ohneda, A., Ishii, S., Horigome, K., Chiba, M., Sakai, T., Kai, Y., Watanabe, K., Yamagata, S.: Effect of intrapancreatic administration of vasoactive intestinal peptide upon the release of insulin and glucagon in dogs. Horm. Metab. Res. 9:447-452 (1977)
17. Kaneto, A., Kaneko, T., Kajinuma, H., Kosaka, K.: Effect of vasoactive intestinal polypeptide infused intrapancreatically on glucagon and insulin secretion. Metabolism 26:781-786 (1977)
18. Makhlouf, G.M., Yau, W.M., Zfass, A.M., Said, S.I., Bodanszky, M.: Comparative effects of synthetic and natural vasoactive intestinal peptide on pancreatic and biliary secretion and on glucose and insulin blood levels in the dog. Scand. J. Gastroent. 13:759-765 (1978)
19. Schebalin, M., Said, S.I., Makhlouf, G.M.: Stimulation of insulin and glucagon secretion by vasoactive intestinal peptide. Am. J. Physiol. 232:E 197-E 200 (1977)
20. Turner, D.S., Etheridge, L., Marks, V., Brown, J.C., Mutt, V.: Effectiveness of the intestinal polypeptides IRP, GIP, VIP and motilin on insulin release in the rat. Diabetologia 10:459-463 (1974)
21. Unger, R.H., Ketterer, H., Dupré, J., Eisentraut, A.M.: The effects of secretin, pancreozymin, and gastrin on insulin and glucagon secretion in anesthetized dogs. J. Clin. Invest. 46:630-645 (1967)
22. Lerner, R.L.: The augmentation effect of secretin on the insulin responses to known stimuli: Specificity for glucose. J. Clin. Endocrinol. Metabol. 45:1-9 (1977)
23. Enk, B.: Secretin induced insulin response. Acta Endocrinol. (Kbh.) 82:312-317 (1976)

24. Fahrenkrug, J., Schaffalitzky de Muckadell, O.B., Kühl, C.: Effect of secretin on basal- and glucose-stimulated insulin secretion in man. Diabetologia 14:229-234 (1978)
25. Desbuquois, B., Landat, M.M., Landat, Ph.: Vasoactive intestinal polypeptide and glucagon: Stimulation of adenylate cyclase activity via distinct receptors in liver and fat cell membranes. Biochem. Biophys. Res. Commun. 53:1187-1194 (1973)
26. Christophe, J.P., Conlon, T.P., Gardner, J.D.: Interaction of porcine vasoactive intestinal peptide with dispersed pancreatic acinar cells from guinea pig. Binding of radioiodinated peptide. J. Biol. Chem. 251:4629-4634 (1976)
27. Rehfeld, J.F., Stadil, F., Malmström, J., Miyata, M.: Gastrin heterogeneity in serum and tissue: A progress report. In: Thompson, J.C. (ed.): Gastrointestinal hormones. University of Texas press, Austin and London, 1975, pp. 43-58
28. McGuigan, J.E.: Gastric mucosal intracellular localization of gastrin by fluorescence. Gastroenterology 55:315-327 (1968)
29. Dubois, P.M., Paulin, C., Chayvialle, J.A.: Identification of gastrin-secreting cells and cholecystokinin-secreting cells in the gastrointestinal tract of the human fetus and adult man. Cell Tiss. Res. 175: 351- 356 (1976)
30. Larsson, L.-I., Rehfeld, J.F., Sundler, F., Håkanson, R.: Pancreatic gastrin in foetal and neonatal rats. Nature 262:609-610 (1976)
31. Uvnäs-Wallensten, K., Rehfeld, J.F., Larsson, L.-I., Uvnäs, B.: Heptadecapeptide gastrin in the vagal nerve. Proc. Natl. Acad. Sci. (USA) 74:5707-5710 (1977)
32. Rehfeld, J.F.: Effect of gastrin and its C-terminal tetrapeptide on insulin secretion in man. Acta endocrinol. (Kbh.) 66:169-176 (1971)
33. Rehfeld, J.F., Stadil, F.: The effect of gastrin on basal- and glucose- stimulated insulin secretion in man. J. Clin. Invest. 52:1415--1426 (1973)
34. Kuzuya, T., Kajinuma, H., Sando, H., Hayashi, M., Ide, T., Kosaka, K.: Comparison of the early time courses of the release of insulin that follow injections of tetragastrin, tolbutamide, xylitol, and glucose into the pancreatic artery of dogs. Diabetes 27:1189-1195 (1978)
35. Grossman, M.I.: Gastrin, cholecystokinin and secretin act on one receptor. Lancet I:1088-1089 (1970)

GIP-like Immunoreactivity in Glucagon Cells
Interactions Between GIP and Glucagon on Insulin Release

Bo Ahrén, Rolf Håkanson, Ingmar Lundquist, Kristina Sjölund and Frank Sundler

Departments of Pharmacology and Histology, University of Lund, Lund, Sweden

SUMMARY

In the present study the cellular and subcellular distribution of immunoreactive glucagon and GIP were studied in the mouse. Furthermore, the effects of pure GIP and glucagon on basal and stimulated insulin secretion were investigated.

Immunohistochemistry revealed that immunoreactive GIP occurred in the pancreatic glucagon cells and in endocrine cells, also displaying glucagon immunoreactivity, scattered along the small and large intestines. Electron immunocytochemistry revealed that the GIP-like material and glucagon coexisted in the secretory granules of the pancreatic glucagon cells.

Pure porcine GIP and glucagon both stimulated basal insulin release. When equipotent doses of the peptides were given together, the two peptides antagonized each other's effect.

Both peptides potentiated glucose- and carbachol-induced insulin release. When equipotent doses of the two peptides were given together prior to the administration of each of these secretagogues their effects on insulin release were additive.

In conclusion, immunoreactive GIP and glucagon coexist in the secretory granules of the pancreatic glucagon cells. Whether this signifies the presence of a peptide with GIP-like bioactivity in the glucagon cells remains to be established. Natural GIP and glucagon antagonize each other's stimulatory effect on basal insulin release. They potentiate glucose- and carbachol-induced insulin secretion, and they show additive influences in this respect.

INTRODUCTION

Pancreatic glucagon as well as GIP (gastric inhibitory polypeptide or glucose-dependent insulinotropic polypeptide) (Brown et al. 1975, Unger &

Orci 1976) are known to stimulate insulin release both *in vivo* in man (Samols et al. 1965, Dupré et al. 1973), dog (Pederson et al. 1975), rat (Taminato et al. 1977) and mouse (Ahrén & Lundquist 1980) as well as *in vitro* (rat) (Grodsky et al. 1967, Taminato et al. 1977, Pederson & Brown 1978, Schäfer & Schatz 1979). GIP is released from the gastrointestinal tract by feeding (Cataland et al. 1974) and is assumed to be the strongest incretin candidate (Creutzfeldt 1979). Glucagon and glucagon-like peptides occur not only in the pancreas but also in the gut (gut-type glucagon, glicentine) and in some species also in the stomach (Larsson et al. 1975, Sundler et al. 1976, Holst 1978).

Recently it was demonstrated that pancreatic and gut glucagon cells of rat, guinea pig, cat, dog, pig, and man in addition to glucagon contain immunoreactive GIP (Smith et al. 1977, Alumets et al. 1978). Nothing is known about the identity and biological activity of the GIP-like peptide. Since glucagon and the GIP-like peptide occur in the same cell type the two peptides may be released together upon stimulation of the cell and they may then jointly influence other cell systems. The possibility that the GIP-like material shares biological activity with natural GIP stimulated a study of the effects on basal and stimulated insulin secretion of natural GIP and interactions between GIP and glucagon. Studies were also undertaken to further clarify the proposed coexistence of immunoreactive GIP and glucagon in the same cell type.

MATERIALS AND METHODS

Animals.

Female mice of the NMRI strain (Lab. Animal Breeding, Laven, Denmark), weighing 25-35 g, were used. The animals were fed standard pellets (Astra-Ewos, Södertälje, Sweden), and tap water *ad libitum* before and throughout the experiments.

Drugs.

Pure porcine GIP was a gift from Professor V. Mutt, Karolinska Institutet, Stockholm, Sweden. It was isolated by the method of Brown et al. (Brown et al. 1970) from a partially purified preparation of cholecystokinin. Also pure porcine vasoactive intestinal polypeptide (VIP) and pure porcine secretin were provided by Professor Mutt. Pure porcine glucagon and glicentine (GLI-1) were kindly donated by Dr. A.J. Moody, Novo Res. Inst., Denmark. Synthetic GIP was kindly supplied by Professor H. Yajima, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

D-glucose and carbachol were from British Drug Houses Ltd., Poole, England.

Light and Electron Microscopy

I. Immunohistochemistry.

The animals were killed by decapitation. Specimens from the pancreas and the intestines were dissected out, frozen to the temperature of liquid nitrogen in a mixture of propane and propylene and freeze-dried. The specimens were then fixed by exposure to gaseous diethylpyrocarbonate at 55°C for 3 hours and embedded in paraffin or Araldite. Sections were cut at 6 µm thickness (paraffin) or 1 µm (Araldite). After removal of the embedding medium the sections were processed for the immunohistochemical demonstration of GIP or glucagon. Four GIP antisera were obtained from different sources (see Table 1). Cross-reactivity with the various peptides of the

Table I

Source and properties of the various GIP antisera.

Code no.	Raised against	Working dilution		Cross-reactivity with				Source (ref.)
		immunofluorescence	PAP	glucagon	glicentine	VIP	secretin	
R-D 11/19/77	Pure porcine GIP	1:80	1:5120	0	0	0	0	Dr. T. O'Dorisio (Alumets et al. 1978) Ohio State Univ., Ohio, USA
RIC-34	Pure porcine GIP-ovalbumin	-	1:200	0	0	0	0	Dr. L. Morgan, (Morgan et al. 1978) Univ. of Surrey, Guilford, England
R-07	Pure porcine GIP-BSA	-	1:50	0	0	0	0	Dr. J.C. Brown (Kuzio et al. 1974) Univ. of Brit. Columbia, Vancouver, Canada
R-65	Crude porcine GIP	-	1:240	n.t.	n.t.	n.t	n.t	Dr. A.J. Moody, (Lauritsen & Moody 1978) Novo Res. Inst. Copenhagen, Denmark

0 no cross-reactivity

n.t. not tested

glucagon family was tested by preincubation of the properly diluted GIP antisera with glucagon, VIP, secretin (100 µg/ml), or glicentine (50 µg/ml) for 24 hours at 4°C. The glucagon antiserum (No 4304) was kindly donated by Dr. J. Holst, Bispebjerg Hospital, Copenhagen, Denmark. This antiserum, which demonstrates both pancreatic and gut-type glucagon, has been characterized in detail elsewhere (Larsson et al. 1975). The site of antigen--antibody reaction was revealed by the indirect immunofluorescence method of Coons et al. (Coons et al. 1955) or by the peroxidase-antiperoxidase (PAP) technique of Sternberger (Sternberger 1974). FITC-labelled and unlabelled goat anti-rabbit IgG was purchased from Statens Bakteriologiska Laboratorium, Stockholm, Sweden. PAP complex was obtained from Cappel Ltd., Downington, Pennsylvania, USA. Staining controls included the use of non-immune rabbit serum instead of the primary or the secondary antibody. For specificity controls we used GIP antiserum exposed to pure porcine or synthetic GIP (10 µg/ml) for 24 hours at 4°C. The corresponding specificity involved the use of pure porcine glucagon (10 µg/ml).

II. Electron Immunocytochemistry.

Mice were perfused for 5 to 10 minutes via the ascending aorta with 0.5% glutaraldehyde and 4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.2, under diethyl ether anesthesia. Small pieces of tissue from the pancreas were rapidly dissected out and immersed in the fixation fluid for 1 to 4 hours. The specimens were post-fixed in 1% OsO_4 for 2 hours, dehydrated in graded ethanol solutions, contrasted *en bloc* for 1/2 hour in a mixture of 1% phosphotungstic acid and 0.5% uranyl acetate in ethanol and embedded in Araldite. Ultra-thin sections were etched in 10% hydrogen peroxide and 1% periodic acid and exposed to GIP antiserum or glucagon antiserum (as above). The site of antigen-antibody reaction was located by PAP staining. Staining and specificity controls were as for immunocytochemistry at the light microscopic level.

Experiments.

I. Effects of GIP or Glucagon on Insulin Release.

Basal Insulin Release. Natural GIP and glucagon were dissolved in 0.9% NaCl with addition of 0.1% gelatine and administered in a tail vein; 10 µl/g body weight was injected; from 0.27 nmol/kg to 4.25 nmol/kg (GIP) and from 0.53 nmol/kg to 8.5 nmol/kg (glucagon) was given. Control animals were injected with saline. Two minutes after the injection 250 µl blood was drawn by orbital puncture. The mice were unanaesthetized throughout the experiments. In initial experiments it was found that the plasma concentration of immunoreactive insulin was maximal two minutes after the intravenous injection of GIP or glucagon.

Stimulated Insulin Release. Natural GIP and glucagon were injected intravenously, 5 µl/g, 15 seconds prior to a rapid intravenous injection of D-glucose or carbachol, 5 µl/g. The doses given were 0.27 or 0.53 nmol/kg (GIP) and 0.53 or 1.06 nmol/kg (glucagon). Controls were injected, with saline and one of the secretagogues, or with saline alone. Two minutes after the last injection blood was collected. This sequence of injection is considered a useful model when studying the interaction of different polypeptides with insulin secretagogues such as glucose and carbachol, since it has earlier been shown that somatostatin inhibits stimulated insulin release in a dose-dependent manner when injected 15 seconds prior to glucose or carbachol (Lundquist et al. 1979). Repeated experiments in this laboratory have shown that the maximal concentration of immunoreactive insulin in mouse plasma following a rapid intravenous injection of these secretagogues is achieved after two minutes. D-glucose and carbachol were administered in doses giving about 25% of their maximal insulin response, respectively, recorded as increase of immunoreactive insulin in plasma after two minutes. These doses were 1.39 mmol/kg for D-glucose and 80 nmol/kg for carbachol, respectively.

II. Effects of GIP plus Glucagon on Insulin Release

Basal Insulin Release. Equipotent doses of natural GIP and glucagon were administered together as a single intravenous injection, and two minutes later blood was drawn. Four different dose levels were selected, ranging from threshold doses to maximal doses.

Stimulated Insulin Release. Natural GIP and glucagon were administered together as a single intravenous injection. 15 seconds later D-glucose or carbachol was given rapidly i.v. Three different dose combinations of GIP and glucagon were used.

Determination of Insulin and Glucose.

The concentrations of immunoreactive insulin, (IRI), in plasma were determined by the technique of Heding (Heding 1966), using ^{125}I-labelled pig insulin and guinea pig anti-pig insulin. Plasma glucose was measured enzymatically (Bruss & Black 1978). The plasma glucose concentrations were 8.7 $\pm$ 0.2 mmol/l in mice not receiving glucose. There was no difference in glucose levels between the groups. Two minutes after glucose injection the plasma glucose concentrations were 11.7 $\pm$ 0.2 mmol/l. There was no difference with regard to plasma glucose levels amongst the glucose-treated groups.

Statistics

Student's t-test was employed for tests of significance. Means and standard error of the means (SEM) are given. Increase in plasma IRI concentrations in response to stimulation (IRI) was calculated by subtracting the plasma

IRI concentrations in the appropriate saline-injected control group, and by calculating the weighted SEM, taking into account SEM of both groups.

RESULTS

Cellular and Subcellular Localization of Immunoreactive GIP and Glucagon.

With three of the four GIP antisera tested immunoreactive GIP could be demonstrated by immunocytochemistry in pancreatic islet cells as well as in scattered endocrine cells in the mucosa of the small and large intestines (Table 2). The immunoreaction was blocked by exposing the antiserum to natural or synthetic GIP but not to glucagon, glicentine, VIP or secretin (Table 1).

Table 2

Immunostaining of pancreatic and gut endocrine cells using the various GIP antisera.

GIP antiserum	immunostaining of		
	duodenal GIP cells	pancreatic glucagon cells	colonic glicentine cells
R-D 11/19/77	+	+	+
RIC-34	+	+	+
R-07	+	+	+
R-65	+	0	0

\+ immunostaining

0 no immunostaining

By immunostaining of consecutive sections it could be shown that the GIP immunoreactive cells in the pancreatic islets were identical with the glucagon cells (Fig. 1). Also the intestinal GIP immunoreactive cells dis-

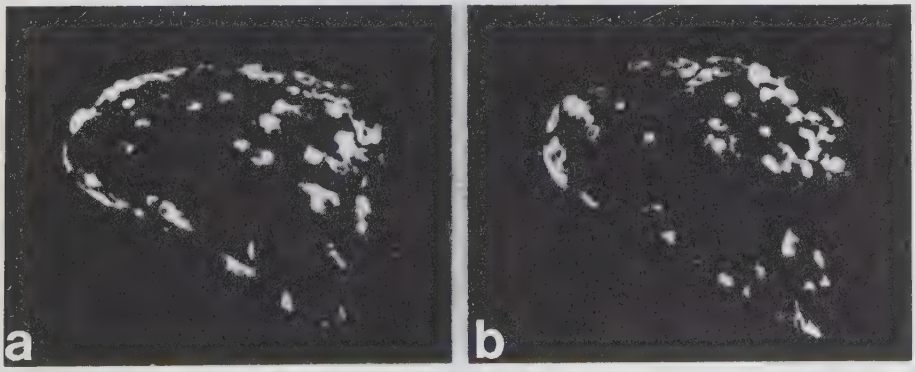


Fig. 1. Mouse pancreas. Two adjacent plastic sections through an islet processed for the immunohistochemical demonstration of glucagon (a) and GIP (b), respectively (immunofluorescence). All glucagon cells display GIP immunofluorescence (X 200). GIP antiserum R-D 11/19/77.

played glucagon immunoreactivity. In the lower small intestines and large intestines both the GIP and the glucagon immunostaining in these cells were intense (Fig. 2).

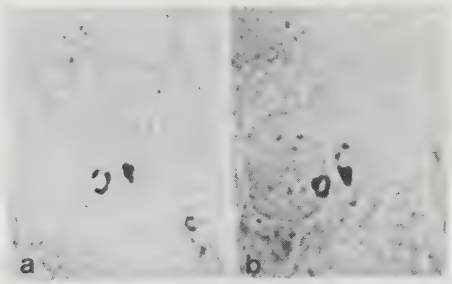


Fig. 2. Mouse colon. Two adjacent plastic sections processed for the immunohistochemical demonstration of glucagon (a) and GIP (b), respectively (immunoperoxidase staining). The GIP immunoreactive cells are identical with those displaying glucagon immunoreactivity (X 300). GIP antiserum R-D 11/19/77.

The fourth antiserum demonstrated GIP immunoreactive cells in the upper small intestine only (Table 2); these cells stained weakly with the glucagon antiserum (Fig. 3). Further, they were identical with the cells stained by the other three GIP antiserum (Fig. 3). The immunoreaction was blocked by adding natural or synthetic GIP to the antiserum.

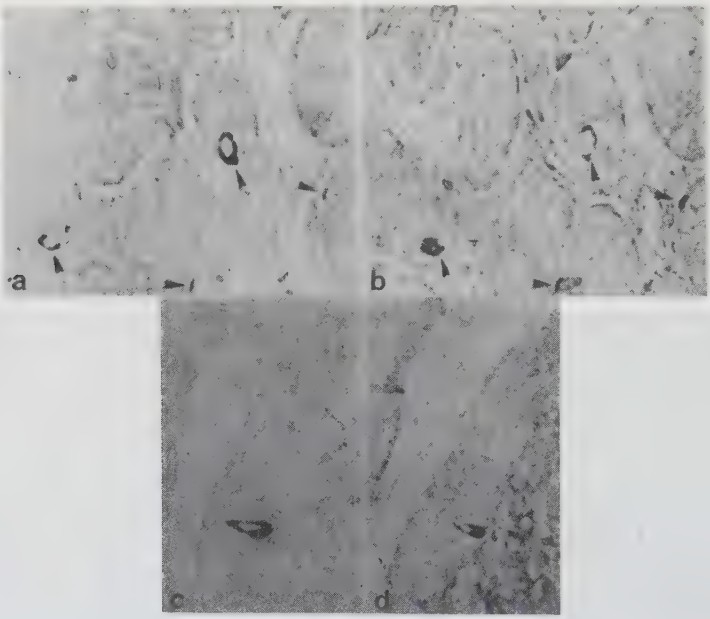


Fig. 3. Mouse duodenum. Four adjacent plastic sections processed for the immunohistochemical demonstration of glucagon (a and c) and GIP (b and d), respectively (immunoperoxidase staining). All GIP immunoreactive cells display glucagon immunoreactivity of varying intensity (X 300). The GIP antiserum (RIC-34) used in (b) demonstrates GIP in glucagon cells in pancreas and gut. The GIP antiserum (R-65) used in (d) demonstrates GIP in glucagon cells of the upper small intestine only.

Immunostaining at the electron microscopic level of material from the pancreas revealed that immunoreactive GIP was localized to the cytoplasmic granules of the pancreatic glucagon cells. The immunoreactive material was evenly distributed over the dense core, in a manner identical with the distribution of glucagon (Fig. 4).

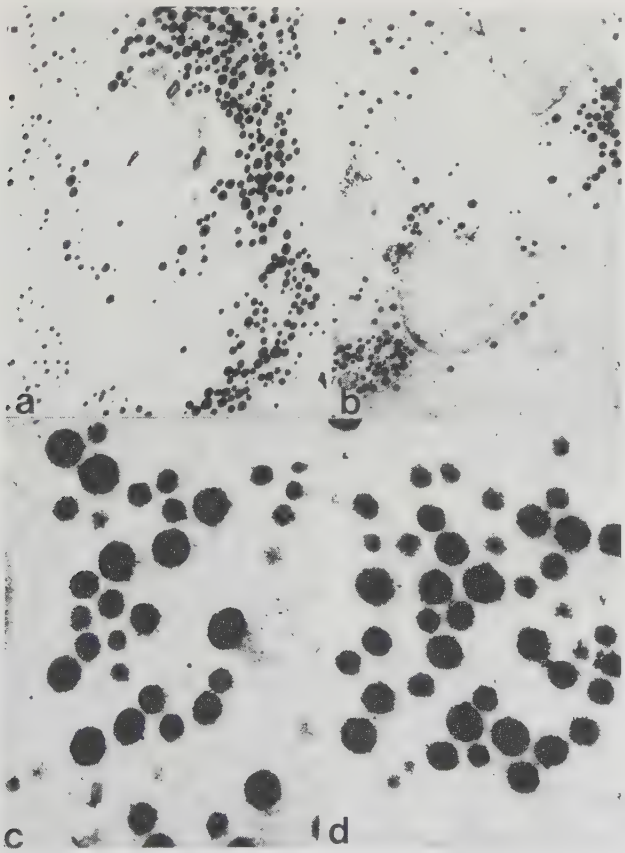


Fig. 4. Immunocytochemical demonstration at the ultrastructural level of GIP (a and c) and glucagon (b and d), respectively, on ultrathin sections of pancreatic glucagon cells (immunoperoxidase staining). Fig. 4 a and b are low magnifications. Fig. c and d show details of cytoplasm in immunoreactive cells. Weak background staining of insulin cell granules in a and b (right part of figs). Heavy deposits of reaction product over all secretory granules in the glucagon cells, indicating coexistence of immunoreactive GIP and glucagon within the granules (a and b x 8000, c and d x 30 000). GIP antiserum R-D 11/19/77.

Effects of Natural GIP and Glucagon on Basal Insulin Secretion

Effects of Natural GIP or Glucagon. Natural GIP or glucagon was injected intravenously in doses from 0.27 nmol/kg to 4.25 nmol/kg (GIP) and from 0.53 nmol/kg to 8.5 nmol/kg (glucagon), respectively. As can be seen in Fig. 5, GIP and glucagon raised plasma IRI in a dose-dependent manner. GIP was slightly more potent in submaximal doses, but the maximal response was the same for the two peptides. In maximal doses they elevated the insulin levels about 40 µU/ml above basal.

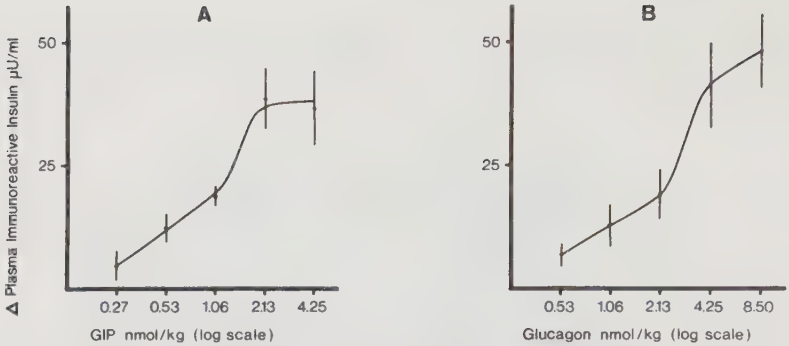


Fig. 5. Increase in plasma immunoreactive insulin concentrations after the i.v. injection of different doses of GIP (Fig. 5 A) or glucagon (Fig. 5 B). Ordinate: increase in plasma IRI (μU/ml). Abscissa: dose of GIP or glucagon (nmol/kg) (log scale). Blood samples were taken two minutes after the injection. There were 19-28 animals in each group. Bars indicate SEMs.

Effects of Natural GIP plus Glucagon. Equipotent doses of the peptides, calculated from the dose-response curves in Fig. 5, were injected together as a single intravenous injection, and plasma IRI was measured in blood samples taken two minutes later. As can be seen in Fig. 6 the resulting increase in plasma IRI was of the same magnitude when the peptides were given together as when each peptide was given alone. Apparently they antagonized each other's effect.

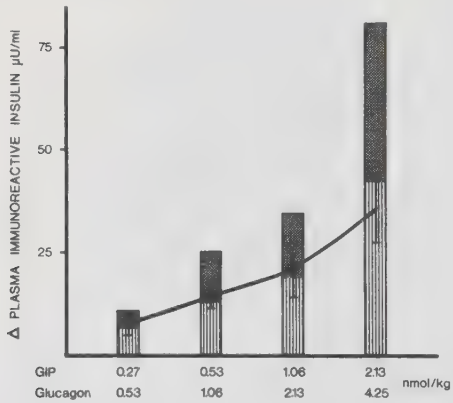


Fig. 6

Increase in plasma immunoreactive insulin concentrations after the i.v. injection of GIP plus glucagon. Blood samples were taken two minutes after the injection. The curve shows the experimental results. The height of the dotted columns indicate the IRI response following injection of GIP alone, and the height of the striped columns following injection of glucagon alone, respectively (data taken from Fig. 5). Thus, the total height of the columns show the expected increase in plasma immunoreactive insulin concentrations if the combined effects of the two peptides were additive. There were 20-29 animals in each experimental group. Bars indicate SEMs.

Effects of Natural GIP and Glucagon on Stimulated Insulin Secretion.

Glucose-Induced Insulin Release. The peptides were injected 15 sec prior to D-glucose.

D-glucose alone raised the plasma insulin levels by 39.6 ± 4.5 μU/ml ($p < 0.001$) (Fig. 7). Both natural GIP and glucagon potentiated this response. A low GIP dose, 0.27 nmol/kg, plus glucose resulted in an increase in plasma IRI of 64.8 ± 6.2 μU/ml ($p < 0.01$ vs glucose injected controls); GIP in a higher dose, 0.53 nmol/kg, plus glucose increased IRI by 80.2 ± 10.5 μU/ml ($p < 0.001$). Also glucagon showed a dose-dependent effect on the glucose-induced insulin release. Glucagon, 0.53 nmol/kg, plus glucose increased the IRI by 66.3 ± 9.2 μU/ml ($p < 0.02$), and glucagon, 1.06 nmol/kg, plus glucose by 84.0 ± 9.9 μU/ml ($p < 0.001$).

When the peptides were given together prior to glucose the increase in IRI was greater than when either of the peptides was given alone. The resulting increase in plasma IRI when 0.27 nmol/kg GIP and 0.53 nmol/kg glucagon plus glucose were given was 91.6 ± 11.9 µU/ml, which is the expected value if the responses to each peptide were additive. Also with 0.53 nmol/kg of GIP and 1.06 nmol/kg of glucagon the peptides appeared to exert additive effects on glucose-induced insulin release (Fig. 7).

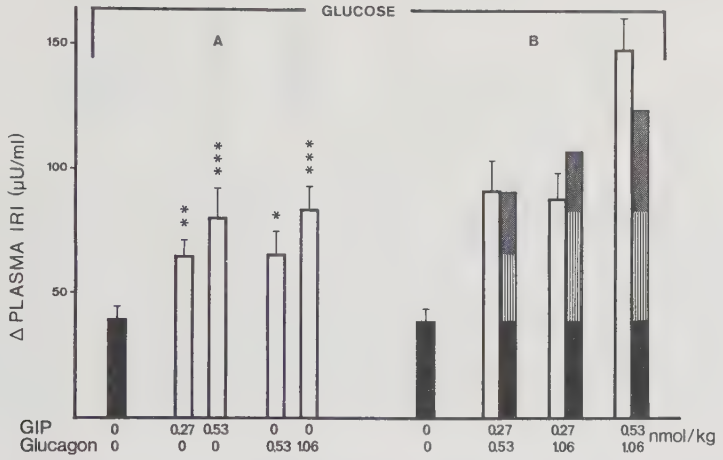


Fig. 7. Increase in plasma immunoreactive insulin concentrations two minutes after the i.v. injection of 1.39 mmol/kg D-glucose. A. Saline (black columns), GIP or glucagon (open columns) was injected 15 seconds prior to glucose. There were 20-33 animals in each group. Bars indicate SEMs. Asterisks indicate probability level of random difference vs the saline glucose-treated-group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

B. GIP plus glucagon was injected i.v. 15 seconds prior to D-glucose (open columns) . Dotted (GIP) and striped (glucagon) columns show the increase of glucose-stimulated insulin release by the two peptides, respectively (data taken from Fig. 7 A). Black columns indicate the increase in immunoreactive insulin concentrations obtained by D-glucose. Thus, the total height of these dotted-striped-black columns indicate the expected increase in immunoreactive insulin concentrations if the combined effects of the two peptides were additive on the glucose-induced insulin release. There were 23-32 animals in each experimental group. Bars indicate SEMs.

Carbachol-Induced Insulin Release. Each of the peptides was injected 15 sec prior to carbachol.

Carbachol alone induced an increase in plasma IRI of 49.5 ± 3.9 µU/ml ($p < 0.001$) (Fig. 8). A low dose of natural GIP and glucagon, respectively, which was found to potentiate glucose-induced insulin release, did not change the plasma insulin response to carbachol. However, when higher doses of each of the peptides were given prior to carbachol it was found that also carbachol-induced insulin secretion was potentiated. GIP, 0.53 nmol/kg, plus carbachol resulted in an increase in IRI of 75.7 ± 8.4 µU/ml ($p < 0.01$ vs the carbachol groups), and the corresponding value for glucagon, 1.06 nmol/kg, plus carbachol was 73.2 ± 6.1 µU/ml ($p < 0.01$).

When the lower doses of GIP and glucagon were given together prior to carbachol the resulting plasma insulin concentrations were higher than in the carbachol-injected control group. When the higher doses of the peptides were injected together prior to carbachol, the increase in plasma IRI reached the value expected if their effects on carbachol induced insulin release were additive (Fig. 8).

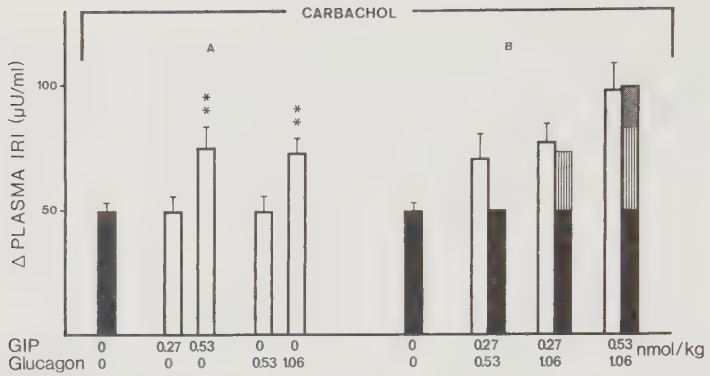


Fig. 8. Increase in plasma immunoreactive insulin concentrations two minutes after the i.v. injection of 80 nmol/kg carbachol. A. Saline (black columns), GIP or glucagon (open columns) was injected 15 seconds prior to carbachol. There were 20-28 animals in each group. Bars indicate SEMs. Asterisks indicate probability level of random difference vs the saline-carbachol-treated group. ** $p < 0.01$.

B. GIP plus glucagon was injected i.v. 15 seconds prior to carbachol (open columns). Dotted (GIP) and striped (glucagon) columns show the increase of carbachol-stimulated insulin release by the two peptides, respectively (date taken from Fig. 8 A). Black columns indicate the increase in immunoreactive insulin concentrations obtained by carbachol. Thus, the total height of these dotted-striped-black columns indicate the expected increase in immunoreactive insulin concentrations if the combined effects of the two peptides were additive on cholinergic-induced insulin release. There were 20-29 animals in each experimental group. Bars indicate SEMs.

DISCUSSION

Several similarities between the 43 amino acid peptide GIP and the 29 amino acid peptide glucagon have been described. In addition to partial chemical homology (Brown & Dryburgh 1971) there are functional similarities in that both peptides have strong effects on gastrointestinal functions and insulin release (cf. Brown et al. 1975, Unger & Orci 1976).

In the present study the distribution of immunoreactive GIP and glucagon in the pancreas and digestive tract of the mouse has been mapped by immunocytochemistry and the effects of natural GIP and glucagon on basal and stimulated insulin secretion have been investigated.

In previous studies GIP was localized to scattered endocrine cells in the upper small intestines (Polak et al. 1973). Immunocytochemical studies have suggested the occurrence of GIP-like material also in the glucagon cells of the pancreas and in cells storing gut-type glucagon (glicentine) in the gut (Smith et al. 1977, Alumets et al. 1978). In the present study four GIP antisera from different sources were used. Three of these antisera demonstrated immunoreactive GIP in pancreatic and gut glucagon cells. The fourth antiserum demonstrated immunoreactive GIP in the duodenum only; these cells stored immunoreactive glucagon as well. Together these findings imply that the immunoreactive GIP in the glucagon cells, though GIP-like, is probably distinct from natural GIP. Also it is notable that attempts to extract GIP from pancreas have failed (Kidd et al. 1979).

Our results indicate that the GIP-like peptide and glucagon are stored together in the secretory granules. This localization makes it likely that

both the GIP-like peptide and glucagon are synthesized within the cell.

The release of GIP as well as glucagon is regulated by glucose (Cataland et al. 1974, Taminato et al. 1977, Pederson & Brown 1978) and also a cholinergic influence has been suggested to play a role in the secretion of these polypeptides (Iversen 1973, Larrimer et al. 1978). Since glucose and the vagus are of importance for the secretion also of insulin we decided to use glucose and the cholinergic agonist carbachol to stimulate insulin secretion. Also, these secretagogues probably stimulate insulin release through different mechanisms (Lundquist et al. 1979).

The present study shows that glucagon as well as GIP stimulate basal insulin release in a dose-dependent manner (cf. Fig. 5). The results show that GIP and glucagon compared to e.g. glucose are weak stimulators of basal insulin secretion and they stimulate insulin secretion at high dose levels only. Earlier, GIP and glucagon have been demonstrated to stimulate insulin release in other species (Samols et al. 1965, Grodsky et al. 1967, Dupré et al. 1973, Taminato et al. 1977, Pederson & Brown 1978). On the other hand, GIP has been shown to be without effect on basal insulin secretion in man when given in doses resulting in plasma concentrations comparable to normal postprandial levels (Pederson et al. 1975), indicating that the insulin releasing effect of GIP in the basal state might be unphysiological. However, immunoreactive GIP exists in the glucagon cells which are situated immediately adjacent to the insulin cells. If the GIP-like peptide possesses GIP-like biological activity its insulin releasing activity in the basal state might be of physiological significance, since the local concentration of the GIP-like peptide in the islets may be high.

The GIP-like peptide and glucagon are stored together within the same islet cells, even in the same secretory granules, and they may therefore be released together upon stimulation. Since the GIP-like peptide may share biological properties with natural GIP, the combined effects of GIP and glucagon on insulin release might be of physiological importance.

Under basal conditions, simultaneous injections of equipotent doses of GIP and glucagon were followed by the same insulin release as injection of each peptide alone. The doses were carefully selected from the dose--response curves; in maximal and threshold doses as well as in doses in the medium dose range the two peptides seemed to antagonize each other's effect on basal insulin secretion. Recently it was reported that GIP and glucagon antagonized each other's effect on glucose-induced insulin release in isolated islets, but the combined effect of the two peptides on basal insulin release was not studied (Schäfer & Schatz 1979).

Interactions between GIP and glucagon have also been demonstrated in fat cells (Dupré et al. 1976, Ebert & Brown 1976). GIP, which by itself is non-lipolytic, displaced glucagon from the receptors and inhibited glucagon-induced lipolysis and cAMP production (Dupré et al. 1976). GIP had no influence on VIP- or secretin-induced lipolysis. Thus, interactions between GIP and glucagon may not be unique for the insulin cells.

GIP and glucagon have been reported to potentiate stimulated insulin release (Samols et al. 1965, Pederson et al. 1975, Taminato et al. 1977, Ahrén & Lundquist 1980) and this study shows that both peptides potentiated glucose- and carbachol-induced insulin release in the mouse. The insulin secretory mechanisms seemed to be more sensitive to the two peptides during a glucose stimulus than during cholinergic stimulation. It may be noted that the two peptides exerted these effects at dose levels that

were without effect on basal insulin secretion. With regard to GIP, the potentiation of glucose- and cholinergically stimulated insulin release might be of importance after feeding, where the GIP level in plasma is high (Cataland et al. 1974), and the insulin release is stimulated by both the vagus (Woods & Porte 1974) and glucose. When the insulin cell was stimulated in vivo the interaction of GIP and glucagon noted in the basal state changed from antagonism to additive synergism. Thus, the insulin secretory response in vivo to simultaneous influences of GIP and glucagon is apparently quite different depending on whether the insulin secretion is stimulated by e.g. glucose or the vagus, or not.

In conclusion, a GIP-like peptide is stored together with gut glucagon (glicentine) in endocrine cells scattered along the small and large intestines. Furthermore, a GIP-like peptide coexists with glucagon in the pancreatic glucagon cells. The GIP-like peptide is stored together with glucagon in the secretory granules. If the GIP-like peptide shares the biological activities with natural GIP is not known and remains to be established. If the GIP-like peptide and glucagon are released jointly upon stimulation of the pancreatic glucagon cell, they may act together on nearby insulin cells.

Natural GIP and glucagon stimulate basal insulin secretion and potentiate glucose- or carbachol-induced insulin release in vivo. Under basal conditions natural GIP and glucagon antagonize each other's insulin-releasing effect, but upon stimulation of the insulin cell by glucose or carbachol this interference changes to an additive synergism.

ACKNOWLEDGEMENTS

The expert technical assistance of Åsa Amilon and Peter Okmark is gratefully acknowledged. This work was supported by the Swedish Medical Research Council (04P-4289, 14X-4286, 04X-4499) and by grants from the Medical Faculty of Lund and from the Swedish Diabetes Association.

REFERENCES

AHREN, B. & LUNDQUIST, I. 1980. Effects of glicentine on insulin secretion. Horm Metab Res 12:582-586.

ALUMETS, J., HÄKANSON, R., O'DORISIO, T., SJÖLUND, K. & SUNDLER, F. 1978. Is GIP a glucagon cell constituent? Histochemistry 58:253-257.

BROWN, J.C. & DRYBURGH, J.F. 1971. A gastric inhibitory polypeptide. II. The complete amino acid sequence. Can J Biochem 49:867-872.

BROWN, J.C., DRYBURGH, J.R., ROSS, S.A. & DUPRÉ, J. 1975. Idenfications and actions of gastric inhibitory polypeptide. Recent Progr Horm Res 31:487-532.

BROWN, J.C., MUTT, V. & PEDERSON, R.A. 1970. Further purification of a polypeptide demonstrating enterogastrone activity. J Physiol 209: 57-64.

BRUSS, M.L. & BLACK, A.L. 1978. Enzymatic microdetermination of glycogen. Analyt Biochem 84:309-312.

CATALAND, S., CROCKETT, S.E., BROWN, J.C. & MAZZAFERRI, E.L. 1974. Gastric inhibitory polypeptide (GIP): Stimulation by oral glucose in normal man. J Clin Endocrinol Metab 39:223-228.

COONS, A.H., LEDUC, E.H. & CONNOLLY, J.M. 1955. Studies on antibody production. I. A method for the histochemical demonstration of specific

antibodies and its application to a study of the hyperimmune rabbit. J Exp Med 102:49-59.
CREUTZFELDT, W. 1979. The incretin concept today. Diabetologia 16:75-85.
DUPRE, J., GREENIDGE, N., MCDONALD, T.J., ROSS, S.A. & RUBINSTEIN, D. 1976. Inhibition of action of glucagon in adipocytes by gastric inhibitory polypeptide. Metabolism 25:1197-1199.
DUPRE, J., ROSS, S.A., WATSON, D. & BROWN, J.C. 1973. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 37:826-828.
EBERT, R. & BROWN, J.C. 1976. Effect of gastric inhibitory polypeptide (GIP) on lipolysis and cyclic AMP levels in isolated fat cells. Europ J Clin Invest 6:327.
GRODSKY, G.M., BENNETT, L.L., SMITH, D.F. & SCHMID, F.G. 1967. Effect of pulse administration of glucose or glucagon on insulin secretion in vitro. Metabolism 16:222-233.
HEDING, L. 1966. A simplified insulin radioimmunoassay method. In: DONATO, L., MILHAUD, G. & SIRCHIS, J. (editors) Labelled proteins in tracer studies. Euratom, Brussels pp. 345-350.
HOLST, J. 1978. Extrapancreatic glucagons. Digestion 17:168-190.
IVERSEN, J. 1973. Effect of acetylcholine on the secretion of glucagon and insulin from the isolated, perfused canine pancreas. Diabetes 22:381-387.
KIDD, G.S., DONOWITZ, M., O'DORISIO, T., CATALAND, S. & NEWMAN, F. 1979. Mild chronic watery diarrhea-hyperkalemia syndrome associated with pancreatic islet cell hyperplasia. Am J Med 66:883-888.
KUZIO, M., DRYBURGH, J., MALLOY, K.M. & BROWN, J.C. 1974. Radioimmunoassay for gastric inhibitory polypeptide. Gastroenterology 66:357-364.
LARRIMER, J.N., MAZZAFERRI, E.L., CATALAND, S. & MEKHJIAN, H.S. 1978. Effects of atropine on glucose-stimulated gastric inhibitory polypeptide. Diabetes 27:638-642.
LARSSON, L.-I., HOLST, J., HÅKANSON, R. & SUNDLER, F. 1975. Distribution and properties of glucagon immunoreactivity in the digestive tract of various mammals: An immunohistochemical and immunochemical study. Histochemistry 44:281-290.
LAURITSEN, K.B. & MOODY, A.J. 1978. The response of gastric inhibitory polypeptide (GIP) and insulin to glucose in duodenal ulcer patients. Diabetologia 14:149-153,
LUNDQUIST, I., SUNDLER, F., ÅHREN, B., ALUMETS, J. & HÅKANSON, R. 1979. Somatostatin, pancreatic polypeptide, substance P, and neurotensin: Cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology 104:832-838.
MORGAN, L.M., MORRIS, B.A. & MARKS, V. 1978. Radioimmunoassay of gastric inhibitory polypeptide. Ann Clin Biochem 15:172-177.
PEDERSON, R.A. & BROWN, J.C. 1978. Interaction of gastric inhibitory polypeptide, glucose, and arginine on insulin and glucagon secretion from the perfused rat pancreas. Endocrinology 103:610-615.
PEDERSON, R.A., SCHUBERT, H.E. & BROWN, J.C. 1975. The insulinotropic action of gastric inhibitory polypeptide. Can J Physiol Pharmacol 53: 217-223.
POLAK, J.M., BLOOM, S.R., KUZIO, M., BROWN, J.C. & PEARSE, A.G.E. 1973. Cellular localization of gastric inhibitory polypeptide in the duodenum and jejunum. Gut 14:284-288.
SAMOLS, E., MARRI, G. & MARKS, V. 1965. Promotion of insulin secretion by glucagon. Lancet 2:415-416.
SCHÄFER, R. & SCHATZ, M. 1979. Stimulation of (pro-)insulin biosynthesis and release by gastric inhibitory polypeptide in isolated islets of rat pancreas. Acta Endocrinol (Kbh) 91:493-500.

SMITH, P.H., MERCHANT, F.W., JOHNSON, D.G., FUJIMOTO, W.Y. & WILLIAMS, R.H. 1977. Immunocytochemical localization of a gastric inhibitory polypeptide-like material within A-cells of the endocrine pancreas. Am J Anat 149:585-590.

STERNBERGER, L.A. 1974. Immunocytochemistry. Prentice-Hall, Englewood Cliffs, New Jersey.

SUNDLER, F., ALUMETS, J., HOLST, J., LARSSON, L.-I. & HÅKANSON, R. 1976. Ultrastructural identification of cells storing pancreatic-type glucagon in dog stomach. Histochemistry 50:33-37.

TAMINATO, T., SEINO, Y., GOTO, Y., INOUE, Y., KADOWAKI, S., MORI, K., NOZAWA, M., YAJIMA, H. & IMURA, H. 1977. Synthetic gastric inhibitory polypeptide. Stimulatory effect on insulin and glucagon secretion in the rat. Diabetes 26:480-484.

UNGER, R.H. & ORCI, L. 1976. Physiology and pathophysiology of glucagon. Physiol Rev 56:778-826.

WOODS, S.C. & PORTE Jr, D. 1974. Neural control of the endocrine pancreas. Physiol Rev 54:596-619.

Horm. Metab. Res. 12 (1980) 582–586

Effects of Glicentine on Insulin Secretion

B. Ahrén and I. Lundquist

Department of Pharmacology, University of Lund, Lund Sweden

Summary

The glucagon-like immunoreactivity of the gastrointestinal tract is heterogeneous, probably including several different peptides. One of these peptides, glicentine, has recently been extracted and highly purified. Furthermore, by immunocytochemistry a glicentine-like peptide has been reported to occur in the glucagon cell of the pancreatic islets.

In the present study we investigated the effects of pure glicentine on insulin release *in vivo* in mice. The effects were compared with effects of two other peptides, glucagon and GIP.

It was found that glicentine had no influence on basal insulin secretion. This was in contrast to equimolar doses of glucagon and GIP, which both stimulated the secretion of insulin. Glucose-induced insulin release was partially inhibited by glicentine. D-glucose, in a dose selected to give a response of 25 % of its maximal, raised the plasma insulin concentrations by 44.0 ± 5.9 μU/ml. The corresponding rise for glicentine plus D-glucose was 22.3 ± 3.7 μU/ml, i.e. glicentine inhibited glucose-induced insulin released by about 50 % ($p < 0.01$). GIP, on the other hand, enhanced glucose-induced insulin release. This enhancement was diminished by glicentine, a reflection of the inhibition by glicentine of the glucose-induced insulin release. Neither glicentine nor GIP in the doses tested had any effect on insulin secretion induced by cholinergic stimulation.

In conclusion, glicentine seems to have no effect on basal insulin release in the mouse, but it partially inhibits glucose-induced insulin secretion. Thus, if the recently demonstrated glicentine-like peptide in the glucagon cell is authentic glicentine, the glucagon cell of the pancreatic islets may contain peptides with stimulatory (glucagon) as well as inhibitory (glicentine) effects on insulin secretion induced by glucose.

Key-Words: *Glucagon-like Immunoreactivity (GLI) – Gut-Type Glucagon (GTG) – Glicentine (GLI–I) – Insulin Secretion*

Introduction

Glucagon stimulates the release of insulin (*Samols, Marri* and *Marks* 1965; *Unger* and *Orci* 1976). The digestive tract in several species contains glucagon; pancreatic-type glucagon (PTG) as well as gut-type glucagon (GTG), usually referred to as glucagon-like immunoreactivity (GLI) (*Unger, Ketterer* and *Eisentraut* 1966; *Larsson, Holst, Håkanson* and *Sundler* 1975). The GLI of gastrointestinal origin seems to be released by glucose (*Samols, Tyler, Marri* and *Marks* 1965; *Unger, Ohneda, Valverde, Eisentraut* and *Exton* 1968; *O'Connor, Conlon, Buchanan* and *Murphy* 1979; *Valverde, Ghiglioni, Matesanz and Casado* 1979). GLI may thus be a possible candidate for the great insulin release following feeding, i.e. the incretin effect, but since the secretion of GTG shows no correlation with insulin release its role as a physiological incretin is not established (*Marco, Baroja, Diaz-Fierros, Villanueva* and *Valverde* 1972; cf. *Holst* 1978).

It has been demonstrated that the GLI of gastrointestinal origin is heterogeneous and can be separated in several peptides (cf. *Holst* 1978). One such peptide, GLI-1 or glicentine, has been highly purified and found to contain 100 amino acids with a molecular weight of 11 625 daltons; its amino acid sequence has been partly clarified (*Jacobsen, Demandt, Moody* and *Sundby* 1977).

Furthermore, it has recently been demonstrated that the glucagon cells in the pancreatic islets can be stained with antisera against glicentine (*Ravazzola, Siperstein, Moody, Sundby, Jacobsen* and *Orci* 1979a). If the glicentine immunoreactivity shares the biological activity of authentic glicentine a paracrine effect of glicentine on insulin release may be of physiological importance. In the present study the effects of pure glicentine on basal as well as stimulated insulin secretion were investigated *in vivo* in mice.

The effects were compared with those of glucagon and GIP. It will be shown that glicentine has no effect on basal insulin secretion, whereas it has an inhibitory action on glucose-stimulated release of insulin.

Materials and Methods

Animals. Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25–35 g, were used. The animals were fed standard pellets (Astra-Ewos, Södertälje, Sweden) and tap water *ad libitum* before and throughout the experiments.

Drugs. Highly purified porcine glicentine was kindly supplied by Dr. A.J. Moody, Novo Research Institute, Copenhagen, Denmark. Porcine GIP was isolated from a partially

Received: 20 Oct. 1979 Accepted: 2 March 1980

0018–5043/80 1132–0582 $ 03.00 © 1980 Georg Thieme Verlag, Stuttgart · New York

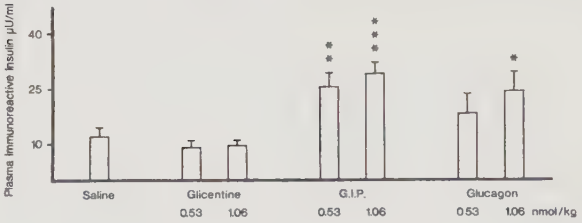


Fig. 1 Plasma immunoreactive insulin 2 min following the i.v. injection of saline, 0.53 or 1.06 nmol/kg of glicentine, GIP or glucagon. There were 10–18 animals in each group. Bars indicate SEMs. The significant level for the difference from the saline-treated control group is indicated by asterisks: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

purified preparation of cholecystokinin (a gift from Professor *V. Mutt*, Karolinska Institutet, Stockholm, Sweden) by the method of *Brown, Mutt* and *Pederson* (1970). Pure porcine glucagon was obtained from Novo Research Institute, Copenhagen, Denmark. All peptides were dissolved in 0.9 % NaCl with the addition of 0.1 % gelatine to avoid adsorption to tubes.

D-glucose and the cholinergic agonist carbachol were from British Drug Houses Ltd., Poole, England. They were dissolved in 0.9 % NaCl.

Experiments

Effects of glicentine, GIP or glucagon on basal insulin release. Each of the three peptides was injected intravenously into a tail vein, 10 µl/g per mouse. The doses given were 0.53 nmol/kg or 1.06 nmol/kg. Control animals were injected with saline. 2 minutes after the injection 250 µl blood was drawn by orbital puncture (*Rerup and Lundquist* 1966). The mice were unanaesthetized throughout. In previous pre-experiments it has been shown that the plasma insulin concentrations were maximal about 2 min after the intravenous injection of GIP or glucagon.

Effects of glicentine and GIP on basal insulin release. GIP was injected intravenously in doses of 0.27 nmol/kg or 0.53 nmol/kg, and the resulting insulin release was compared with the release obtained by injection of 0.27 nmol/kg GIP together with 0.53 nmol/kg glicentine. Blood was collected 2 minutes after the injection.

Effects of glicentine and/or GIP on stimulated insulin release. Glicentine, 0.53 nmol/kg, and GIP, 0.27 nmol/kg, were injected i.v. alone or together, 5 µl/g mouse, 15 seconds prior to a rapid intravenous injection of D-glucose or carbachol, 5 µl/g mouse. Blood was drawn 2 min after the latter injection. Repeated experiments in this laboratory have shown that the maximal concentration of insulin in mouse plasma following a rapid intravenous injection of these secretagogues is achieved after 2 min. D-glucose and carbachol were administered in doses giving about 25 % of the maximal insulin response. These doses were 1.39 mmol/kg for D-glucose and 80 nmol/kg for carbachol, respectively.

Determination of insulin. The concentrations of immunoreactive insulin (I.R.I.) in plasma were determined by the technique of *Heding* (1966), using ^{125}I-labelled pig insulin and guinea pig anti-pig insulin.

Statistics. Student's t-test was employed for tests of significance. Means and standard error of the means (SEM) are given. Increase in plasma IRI concentrations in response to stimulation (ΔIRI) was calculated by subtracting the plasma IRI concentrations in the appropriate saline-injected control group, and by calculating the weighted SEM, taking into account SEM of both groups.

Results

Effects of glicentine, GIP or glucagon on basal insulin release

From Fig. 1 it can be seen that GIP as well as glucagon induced a rise in plasma IRI. GIP enhanced IRI when injected in a dose of 0.53 nmol/kg as well as in a dose of 1.06 nmol/kg. The latter dose resulted in an increase in plasma IRI from 12.0 ± 2.4 µU/ml to 29.2 ± 3.1 µU/ml ($p < 0.001$). Glucagon induced an insulin secretion only in the higher dose, 1.06 nmol/kg, where the insulin concentration was elevated to 24.3 ± 5.2 µU/ml ($p < 0.05$).

In order to compare the effects of glicentine on the insulin release with that of GIP and glucagon, equimolar doses of this peptide were given. The injection of 0.53 or 1.06 nmol/kg of glicentine did not change the plasma IRI concentration. The IRI levels were 8.6 ± 2.6 µU/ml and 9.6 ± 2.0 µU/ml, respectively, which is not different from the saline-treated group. Thus, in contrast to GIP and glucagon, glicentine did not stimulate insulin secretion.

Effects of glicentine and GIP on basal insulin release

GIP was injected intravenously in doses of 0.27 nmol/kg or 0.53 nmol/kg, respectively. As can be seen in Fig. 2 the higher dose stimulated basal insulin release, while the lower one had no effect. The lower dose of GIP resulted in an IRI concentration of 15.6 ± 3.0 µU/ml, compared with 10.8 ± 2.0 µU/ml for the control group (n.s.). To test whether a simultaneous injection of 0.53 nmol/kg of glicentine together with a low dose of GIP, 0.27 nmol/kg, would induce insulin release, a group of mice was treated with this combination. The insulin level in the group treated with the combination was 14.0 ± 3.2 µU/ml (n.s.).

Effects of glicentine and/or GIP on glucose-stimulated insulin release

The injection of D-glucose was followed by an elevation in the concentration of IRI of 44.0 ± 5.9 µU/ml above that of the saline-treated animals ($p < 0.001$). Glicentine or GIP was injected 15 sec prior to glucose to examine their effect on this glucose-induced insulin secretion. The doses of peptides selected for these experiments were 0.27 nmol/

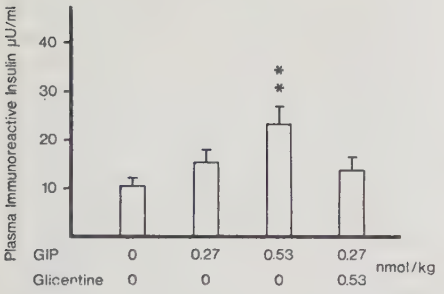


Fig. 2 Plasma immunoreactive insulin 2 min following the i.v. injection of saline, 0.27 nmol/kg or 0.53 nmol/kg GIP and the combination of 0.27 nmol/kg GIP and 0.53 nmol/kg glicentine. There were 12–19 animals in each group. Bars indicate SEMs. The significant level for the difference from the saline-treated control group is indicated by asterisks: $^{**}p < 0.01$.

kg for GIP and 0.53 nmol/kg for glicentine. As shown above (Figs. 1 and 2) these doses did not influence basal insulin secretion. It was found, as can be seen in Fig. 3, that glicentine *inhibited* the glucose-induced insulin release. The increase in plasma IRI following the injections of glicentine and D-glucose was reduced to 11.3 ± 3.7 µU/ml ($p < 0.01$ vs the glucose-treated group). By contrast GIP *potentiated* the glucose-induced insulin secretion. The GIP-

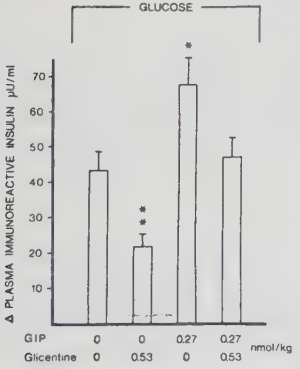


Fig. 3 Increase in plasma immunoreactive insulin above basal levels 2 min following the i.v. injection of D-glucose. Saline, 0.53 nmol/kg glicentine, 0.27 nmol/kg GIP or the combination of 0.53 nmol/kg glicentine and 0.27 nmol/kg GIP was injected 15 sec prior to D-glucose. There were 14–16 animals in each group. Bars indicate SEMs. The significant level for the difference from the saline-glucose treated control group is indicated by asterisks: $^{*}p < 0.05$; $^{**}p < 0.01$.

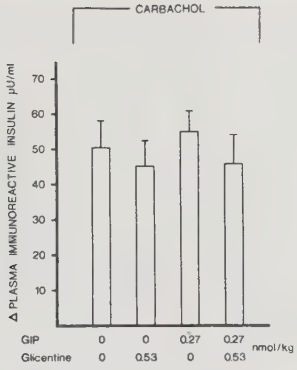


Fig. 4 Increase in plasma immunoreactive insulin above basal levels 2 min following the i.v. injection of carbachol. Saline, 0.53 nmol/kg glicentine, 0.27 nmol/kg GIP or the combination of 0.53 nmol/kg glicentine, and 0.27 nmol/kg GIP was injected 15 sec prior to carbachol. There were 14–16 animals in each group. Bars indicate SEMs.

glucose treated group showed an increase in IRI of 68.0 ± 9.9 µU/ml ($p < 0.05$) above the controls. In another group of mice, GIP, 0.27 nmol/kg, and glicentine, 0.53 nmol/kg, were injected together 15 sec prior to glucose to study whether glicentine influenced the GIP-induced enhancement of the glucose-induced insulin release. The resulting mean increase in plasma IRI was 48.3 ± 7.3 µU/ml above the controls. This might give the impression that glicentine inhibited the effect of GIP on glucose-induced insulin release, but it more probably reflects the inhibition by glicentine of the glucose-induced insulin release. When comparing the further elevation of insulin concentration in response to the peptides on the glucose-induced secretion it is seen that GIP alone enhanced the IRI in plasma by 24 µU/ml and that GIP plus glicentine increased IRI by 26 µU/ml.

Effects of glicentine and/or GIP on carbachol-stimulated insulin release

In this experiment we studied the effects of glicentine, GIP or the combination of both on cholinergic insulin release. As can be seen in Fig. 4 the injection of carbachol was followed by an increase in the IRI concentrations of 51.2 ± 8.2 µU/ml ($p < 0.001$) above the controls. Neither the injection of 0.27 nmol/kg GIP, 0.53 nmol/kg glicentine nor the injection of peptides in combination 15 sec prior to carbachol injection altered the insulin response to carbachol.

Discussion

The existence of several different forms of glucagon has been postulated on the basis of immuno-

chemical studies and, to complicate matters further, pancreatic-type glucagon has recently been found in the gastrointestinal tract (*Larsson* et al. 1975; *Sundler, Alumets, Holst, Larsson* and *Håkanson* 1976, cf. *Holst* 1978). When immunizing with pancreatic-type glucagon, different kinds of antisera are obtained. They are called "specific" and "non-specific" antisera, respectively. The "specific" antibodies seem to be directed against the C-terminal sequence of glucagon (*Assan and Slusher* 1972) and react specifically with pancreatic-type glucagon (PTG), which may also be of extrapancreatic origin. The "non-specific" antibodies, on the other hand, will react with several peptides, including PTG. Peptides reacting with these antibodies and not with the "specific" ones are called GTG, gut-type glucagon (cf. *Holst* 1978).

The GTG's can be separated by gel filtration into two main peaks (cf. *Holst* 1978) with molecular weights of about 10000–12000 and 3000–4000 daltons, respectively. The low-molecular peak, peak 2, has been shown to stimulate insulin release *in vivo* (*Valverde, Rigopoulu, Exton, Ohneda, Eisentraut* and *Unger* 1968; *Ohneda, Horigome, Kai, Habashi, Ishii* and *Yamagata* 1976) and in rat pancreas pieces (*Gutman, Fink, Voyles, Selawry, Penhos, Lepp* and *Recant* 1973). In the isolated perfused rat pancreas, however, no insulin-releasing activity of this peak has been found (*Tanaka, Matsuyama, Shima, Sawazaki, Tarui* and *Kumahara* 1977). The reason for this discrepancy is not known.

In contrast to peak 2, extracts of peak 1 have not been found to stimulate basal insulin release *in vivo* (*Ohneda* et al. 1976), and no effect has been found in the isolated rat pancreas (*Tanaka* et al. 1977). In rat pancreas pieces, however, peak 1 stimulated insulin secretion, but the effect was not suppressed by the "non-specific" glucagon antisera and thus probably not dependent on GTG (*Gutman* et al. 1973). Experiments with these two parts of the GTG thus suggest an insulin-releasing activity in the low-molecular peak. The studies have, however, not been undertaken with pure peptides and may therefore be misleading.

Glicentine, a peptide belonging to the GTG group of peptides, has been isolated and purified. It has been suggested that glicentine is a prohormone to glucagon (*Jacobsen* et al. 1977; *Ravazzola, Siperstein, Moody, Sundby, Jacobsen* and *Orci* 1979b) or to other peptide fragments with glycogenolytic properties (*Holst* 1978). By itself it has been found not to bind to the glucagon receptor of the liver cell membrane nor to stimulate the glucose production of isolated hepatocytes (cf. *Holst* 1978). Glicentine seems to belong to the factors secreted during glucose absorption (*Valverde* et al. 1979), but plasma immunoreactive glicentine is not increased by oral glucose intake in man (*Moody, Sundby, Jacobsen* and *Lauritsen* 1978).

It has recently been demonstrated that the glucagon cell of the pancreas contains a glicentine-like peptide, which is located in the secretory granules (*Ravazzola* et al. 1979a).

In the present study it was shown that glicentine had no effect on basal insulin secretion *in vivo* in the mouse, but it inhibited glucose-induced insulin release. The effect of glicentine was compared with that of GIP and glucagon. The two latter hormones are known to induce release of insulin (*Samols, Marri* and *Marks* 1965; *Pederson* and *Brown* 1976) and both immunoreactive GIP and immunoreactive glicentine have been shown to occur in the glucagon cell of the pancreatic islets (*Smith, Merchant, Johnson, Fujimoto* and *Williams* 1977; *Alumets, Håkanson, O'Dorisio, Sjölund* and *Sundler* 1978; *Ravazzola* et al. 1979b). GIP in a low dose that does not evoke insulin secretion potentiated the glucose-induced insulin release, which confirms previous results on the glucose-dependence of the insulin releasing effect of GIP (*Pederson* and *Brown* 1976). Neither GIP nor glicentine in the doses tested influenced the cholinergically stimulated insulin cell.

The mechanism behind the inhibitory action of glicentine on glucose-induced insulin secretion is not known. Its effect resembles that of the islet peptide pancreatic polypeptide, which also inhibits glucose-induced insulin release, while having no effect on insulin secretion induced by carbachol (*Lundquist, Sundler, Ahrén, Alumets* and *Håkanson* 1979). This is in contrast to somatostatin which inhibits release of insulin stimulated with either of the two secretagogues (*Lundquist* et al. 1979).

The present study thus demonstrates that glicentine inhibits glucose-induced insulin release and has no effect on basal plasma IRI. If the glicentine-like peptide in the A_2 (glucagon) cells (*Ravazzola* et al. 1979a) is authentic glicentine, which remains to be established, this cell type contains both stimulatory and inhibitory peptides with regard to insulin secretion.

Acknowledgements

The expert technical assistance of Asa Amilon and Peter Okmark is gratefully acknowledged.
This investigation was supported by the Swedish Medical Research Council (04P-4289, 14X-4286) and by grants from the Medical Faculty, University of Lund, Lund, Sweden and the Swedish Diabetes Association.

References

Alumets, J., R. Håkanson, T. O'Dorisio, K. Sjölund, F. Sundler: Is GIP a glucagon cell constituent? Histochemistry 58: 253–257 (1978)

Assan, R., N. Slusher: Structure/function and structure/immunoreactivity relationships of the glucagon molecule and related synthetic peptides. Diabetes 21: 843–855 (1972)

Brown, J.C., V. Mutt, R.A. Pederson: Further purification of a polypeptide demonstrating enterogastrone activity. J. Physiol. 209: 57–64 (1970)

Gutman, R.A., G. Fink, N. Voyles, H. Selawry, J.C. Penhos, A. Lepp, L. Recant: Specific biologic effects of intestinal glucagon-like materials. J. Clin. Invest. 52: 1165–1175 (1973)

Heding, L.: A simplified insulin radioimmunoassay method, in: Labelled Proteins in Tracer Studies. Donato, L., G. Milhaud, J. Sirchis (eds.). Euratom, Brussels (1966). pp 345–350

Holst, J.J.: Extrapancreatic glucagons. Digestion 17: 168–190 (1978)

Jacobsen, H., A. Demandt, A.J. Moody, F. Sundby: Sequence analysis of porcine gut GLI-1. Biochim. Biophys. Acta 493: 452–459 (1977)

Larsson, L.-I., J. Holst, R. Håkanson, F. Sundler: Distribution and properties of glucagon immunoreactivity in the digestive tract of various mammals: An immunohistochemical and immunochemical study. Histochemistry 44: 281–290 (1975)

Lundquist, I., F. Sundler, B. Ahrén, J. Alumets, R. Håkanson: Somatostatin, pancreatic polypeptide, substance P and neurotensin: cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology 104: 832–838 (1979)

Marco, J., I.M. Baroja, M. Diaz-Fierros, M.L. Villanueva, I. Valverde: Relationship between insulin and gut glucagon-like immunoreactivity (GLI) secretion in normal and gastrectomized subjects. J. Clin. Endocrinol. Metab. 34: 188–191 (1972)

Moody, A.J., F. Sundby, H. Jacobsen, K.B. Lauritsen: The tissue distribution and plasma levels of glicentin (gut GLI-I). Scand. J. Gastroenterol. 13: 128 (suppl 49) (1978)

O'Connor, F.A., J.M. Conlon, K.D. Buchanan, R.F. Murphy: The use of perfused rat intestine to characterise the glucagon-like immunoreactivity released into serosal secretions following stimulation by glucose. Horm. Metab. Res. 11: 19–23 (1979)

Ohneda, A., K. Horigome, Y. Kai, H. Itabashi, S. Ishii, S. Yamagata: Purification of canine gut glucagon-like immunoreactivity (GLI) and its insulin releasing activity. Horm. Metab. Res. 8: 170–174 (1976)

Pederson, R.A., J.C. Brown: The insulinotropic action of gastric inhibitory polypeptide in the perfused isolated rat pancreas. Endocrinology 99: 780–785 (1976)

Ravazzola, M., A. Siperstein, A.J. Moody, F. Sundby, H. Jacobsen, L. Orci: Glicentin immunoreactive cells – their relationship to glucagon-producing cells. Endocrinology 105: 499–508 (1979a)

Ravazzola, M., A. Siperstein, A.J. Moody, F. Sundby, H. Jacobsen, L. Orci: Glicentin – precursor of glucagon. Life Sci. 25: 287–290 (1979b)

Rerup, C., I. Lundquist: Blood glucose level in mice. I. Evaluation of a new technique of multiple serial sampling. Acta Endocrinol. (Kbh) 52: 357–367 (1966)

Samols, E., G. Marri, V. Marks; Promotion of insulin secretion by glucagon. Lancet 1965/II, 415–416

Samols, E., J. Tyler, G. Marri, V. Marks: Stimulation of glucagon secretion by oral glucose. Lancet 1965/II, 1257–1259

Smith, P.H., F.W. Merchant, D.G. Johnson, W.Y. Fujimoto, R.H. Williams: Immunocytochemical localization of a gastric inhibitory polypeptide-like material within A-cells of the endocrine pancreas. Am. J. Anat. 149: 585–590 (1977)

Sundler, F., J. Alumets, J. Holst, L.-I. Larsson, R. Håkanson: Ultrastructural identification of cells storing pancreatic-type glucagon in dog stomach. Histochemistry 50: 33–37 (1976)

Tanaka, R., T. Matsuyama, K. Shima, N. Sawazaki, S. Tarui, Y. Kumahara: Insulin releasing activity of gastrointestinal glucagon-like immunoreactive materials in perfused rat pancreas. Endocrinol. Japon. 6: 575–579 (1977)

Unger, R.H., H. Ketterer, A.M. Eisentraut: Distribution of immunoassayable glucagon in gastrointestinal tissues. Metabolism 15: 865–867 (1966)

Unger, R.H., A. Ohneda, I. Valverde, A.M. Eisentraut, J. Exton: Characterization of the response of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. J. Clin. Invest. 47: 48–65 (1968)

Unger, R.H., L. Orci: Physiology and pathophysiology of glucagon. Physiol. Rev. 56: 778–826 (1976)

Valverde, I., M. Ghiglioni, R. Matesanz, S. Casado: Chromatographic pattern of gut glucagon-like immunoreactivity (GLI) in plasma before and during glucose absorption. Horm. Metab. Res. 11: 343–346 (1979)

Valverde, I., D. Rigopoulou, J. Exton, A. Ohneda, A. Eisentraut, R.H. Unger: Demonstration and characterization of a second fraction of glucagon-like immunoreactivity in jejunal extracts. Am. J. Med. Sci. 255: 415–420 (1968)

Requests for reprints should be addressed to: Dr. Bo Ahrén, Department of Pharmacology, University of Lund, Sölvegatan 10, S-22362 Lund (Sweden)

0013-7227/79/1043-0832$02.00
Endocrinology
Copyright © 1979 by The Endocrine Society
Vol. 104, No. 3
Printed in U.S.A.

Somatostatin, Pancreatic Polypeptide, Substance P, and Neurotensin: Cellular Distribution and Effects on Stimulated Insulin Secretion in the Mouse*

INGMAR LUNDQUIST, FRANK SUNDLER, BO AHRÉN, JAN ALUMETS, AND ROLF HÅKANSON

Departments of Pharmacology and Histology, University of Lund, Lund, Sweden

ABSTRACT. Somatostatin, pancreatic polypeptide (PP), substance P, and neurotensin were investigated in mice with regard to their cellular localization in gut and pancreas and their effects on stimulated insulin secretion.

Immunohistochemical demonstration of the different peptides revealed the following distribution. PP cells were confined to the pancreatic islets where they seemed to be in close association with the glucagon cells. They were especially abundant in the duodenal part of the pancreas. Somatostatin cells were found in pancreatic islets as well as throughout the gastrointestinal tract. In contrast to PP cells, somatostatin and glucagon cells were more numerous in the splenic part of the pancreas. Scattered endocrine cells displaying substance P immunoreactivity were found mainly in the duodenum, whereas neurotensin cells predominated in the jejuno-ileum. In addition, substance P immunoreactivity was demonstrated in nerves of the intestinal wall. Neither substance P cells nor neurotensin cells could be demonstrated in the pancreas.

At two dose levels, the different peptides administered iv immediately before iv injection of half-maximal doses of glucose or carbachol produced the following effects. Somatostatin, PP, and a low dose level of substance P inhibited glucose-induced insulin release. At a higher dose level, the inhibiting effect of substance P was abolished. Neurotensin had no effect. Somatostatin inhibited insulin secretion induced by carbachol, whereas substance P and PP were without effect. Neurotensin potentiated the response to carbachol. The results suggest that caution should be exercised when interpreting the effects of the different peptides on stimulated insulin release, since their actions are dependent upon the nature of the stimulus. (*Endocrinology* **104:** 832, 1979)

POLYPEPTIDE hormones originating from the gut and pancreas have long been known to be of importance for the regulation of mammalian fuel digestion and metabolism. Among these hormones are gastrin, secretin, and cholecystokinin in the gut and insulin and glucagon in the pancreatic islets. During the last few years this list has grown considerably. A number of new peptide hormone candidates, some of which were originally detected in the central nervous system, have been demonstrated in the gastro-entero-pancreatic region (for recent reviews see Refs. 1, 2). Among such peptides are somatostatin, substance P, motilin, vasoactive intestinal peptide (VIP), gastric inhibitory peptide (GIP), neurotensin, and pancreatic polypeptide (PP). Somatostatin (3–6), motilin (7–9), GIP (10), neurotensin (11, 12), and PP (13–15) seem to occur exclusively in endocrine cells of the mammalian gut and/or pancreas, whereas substance P occurs in nerves as well (16, 17) and VIP in nerves only (18). The elucidation of the functional role of most of these polypeptides is still largely in its initial stage, and is complicated by species differences in the distribution of their cells of origin (19–21).

The aim of the present investigation was to assess the influence of some of these new pancreatic and gut polypeptides on stimulated insulin secretion in the mouse, and to relate these findings to the occurrence and distribution of the cells from which the peptides originate. The polypeptides selected for this study were the 14 amino acid peptide somatostatin, the 13 amino acid peptide neurotensin, the 11 amino acid peptide substance P, and the 36 amino acid peptide PP, all of which have been reported to occur in the abdominal endocrine organ of different species (3–6, 11–17).

Received November 4, 1977.

Address all correspondence and requests for reprints to: Dr. I. Lundquist, Department of Pharmacology, Sölvegatan 10, S-223 62 Lund, Sweden.

* This work was supported by the Swedish Medical Research Council (Projects 14X-4286, O4P-4289, and O4X-4477), the Swedish Diabetes Association, and the Medical Faculty, University of Lund, Sweden.

Materials and Methods

Animals

Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25–35 g, were used. The animals were kept on a standard pellet diet (Astra-Ewos, Söder-

tälje, Sweden) and tap water *ad libitum* before and throughout the experiments.

Antisera

Antiserum against bovine insulin, raised in guinea pigs, was a gift from Dr. Lise G. Heding, Novo (Copenhagen, Denmark). It was used at a dilution of 1:160. Rabbit antiserum against porcine glucagon was generously donated by Dr. J. J. Holst, Bispebjerg Hospital (Copenhagen, Denmark). It was used at a dilution of 1:20. Antiserum against somatostatin was supplied by Dr. M. P. Dubois, Nouzilly (France). It was used at a dilution of 1:40. Antiserum against bovine PP was given to us by Dr. R. E. Chance, Eli Lilly Co. (Indianapolis, IN). It was used at a dilution of 1:80. Antiserum against substance P was placed at our disposal by Dr. G. Nilsson, Karolinska Institute (Stockholm, Sweden). It was used at a dilution of 1:20. Antiserum against neurotensin was donated by Dr. R. Carraway, Harvard (Boston, MA). It was used at a dilution of 1:40 [for details see Sundler *et al.* (12)]. Unlabeled and fluorescein isothiocyanate-labeled goat antisera against rabbit immunoglobulin G or against guinea pig immunoglobulin G were purchased from Statens Bakteriologiska Laboratorium (Stockholm, Sweden). Peroxidase-antiperoxidase complex was obtained from Cappel Labs. (Downington, PA).

Immunohistochemistry

Tissue specimens were collected from various parts of the pancreas and digestive tract of the mouse. The specimens were frozen to the temperature of liquid nitrogen in a mixture of propane and propylene. After freeze-drying, the specimens were fixed with formaldehyde gas or diethylpyrocarbonate gas and embedded in paraffin or Araldite. Paraffin sections were cut at 5 μm and plastic sections at 1 μm. After removal of the embedding medium, the sections were incubated with either of the antisera for 3 h at room temperature. The sections were rinsed thoroughly, and the site of antigen-antibody reaction was revealed by immunofluorescence or immunoperoxidase (peroxidase-antiperoxidase complex) staining. Details on the immunohistochemical procedures were previously reported (15, 18). Fluorescence was examined with a Leitz Orthoplan fluorescence microscope, with peak excitation at 490 nm (filter setting, 3).

Experimental

Somatostatin, neurotensin, and substance P were purchased from Beckman Bioproducts (Geneva, Switzerland). Bovine PP was generously supplied by Dr. R. E. Chance, Eli Lilly Co. (Indianapolis, IN). Carbachol and D-glucose were obtained from British Drug Houses Ltd. (Poole, England). The polypeptides were dissolved in 0.9% NaCl-0.2% gelatine and administered iv in a tail vein (0.15 ml/30 g BW) at a dose of 4.25 or 42.5 nmol/kg BW 15 sec before the rapid iv injection of a half-maximal dose of the insulin secretagogues, D-glucose (2.78 mmol/kg) or carbachol (0.16 μmol/kg). Blood sampling (250 μl) was performed by the orbital puncture technique in the unanesthetised mouse, as previously described (22). Plasma immunoreactive insulin levels (peak levels of the first phase of the insulin secretory response) were recorded 2 min after the injection of the secretagogues. Repeated experiments in this laboratory have show that maximum concentration of immunoreactive insulin i mouse plasma after a rapid iv injection of D-glucose or carbach is achieved after about 2 min. The concentration of insulin i plasma was determined by the method of Heding (23) usin ^{125}I-labeled pig insulin and guinea pig antipig insulin. Student *t* test was employed for tests of significance.

Results

Distribution and cellular localization

The distribution of endocrine cell types within th pancreatic islets was studied using consecutive semithi plastic sections stained with antisera against insulin glucagon, somatostatin, and PP, respectively (Fig. 1). P cells were more numerous in islets of the duodenal por tion of the pancreas than in those of the splenic portion for glucagon and somatostatin cells, the situation was th reverse. Insulin cells were invariably located in the cente of the islets. Glucagon, somatostatin, and PP cells wer predominantly found at the islet periphery but wer occasionally also found more centrally. At times, gluca gon and PP cells seemed closely associated (Fig. 2 Somatostatin cells were observed throughout the gu whereas cells storing pancreatic-type glucagon and P could not be demonstrated. Cells displaying substance immunoreactivity were found scattered in the epitheliur of both small and large intestines. They were particularl numerous in the duodenum (Fig. 3A). Substance P-lik immunoreactivity was observed also in nerves in th smooth muscle layer throughout the intestines, predom inantly in the colon. They were particularly numerous i the myenteric plexa.

Neurotensin cells were predominantly found in th epithelium of the jejunum and ileum where they occurre disseminated on the villi (Fig. 3B). Occasionally a fe neurotensin cells were also found in the colon.

Neither substance P nor neurotensin was detected i the pancreas of the mouse.

Effects on stimulated insulin secretion

An attempt was made to mimic the possible humora or paracrine influences of the polypeptides on insuli secretory mechanisms. Conceivably, such peptides ma reach the insulin-secreting cells at an earlier stage tha glucose or other nutrient secretagogues. Therefore, it wa decided to inject each of the peptides 15 sec befor glucose. A cholinergic agonist, carbachol, was used as a alternative secretagogue, since it is chemically very di ferent from glucose and presumably has a different mod of action on the insulin-secreting cell.

Ancillary studies revealed that injection of the diffe ent polypeptides at a dose of 4.25 or 42.5 nmol/kg di not significantly change basal insulin levels in the mous

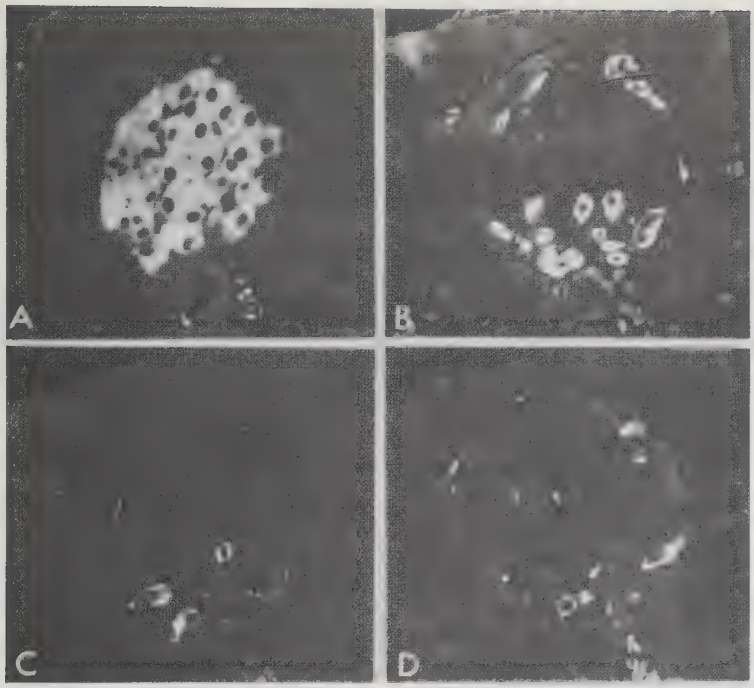


FIG. 1. Distribution of the various endocrine cell types in a pancreatic islet of the mouse. Splenic portions, formaldehyde fixation. Consecutive plastic sections were stained by immunofluorescence. A, Insulin cells; B, glucagon cells; C, PP cells; D, somatostatin cells (×400).

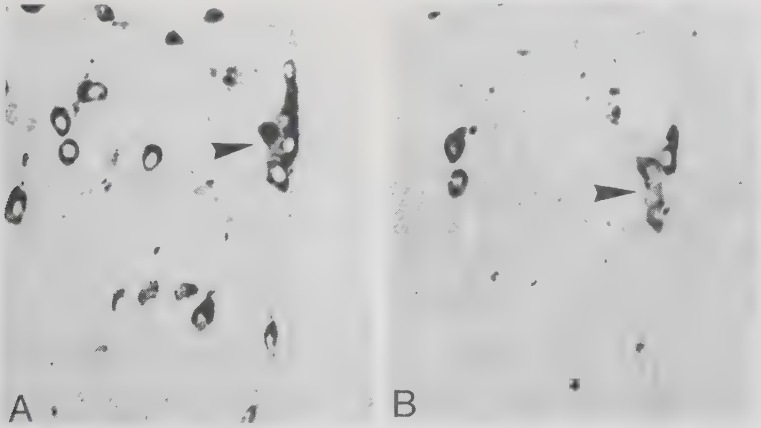


FIG. 2. Glucagon cells (A) and PP cells (B) in islet in the splenic lobe of mouse pancreas consecutive plastic sections. Note the apparent association between PP cell clusters (arrows) and glucagon cells (×300).

during the 2-min interval studied. The first series of experiments was designed to test the effects of somatostatin and PP (4.25 nmol/kg) on insulin release induced by glucose or carbachol. Figure 4 shows that somatostatin suppressed insulin release induced by both glucose and carbachol by about 70%, whereas PP displayed a moderate inhibitory effect on glucose-stimulated insulin release (about 40% decrease) but had no effect on insulin

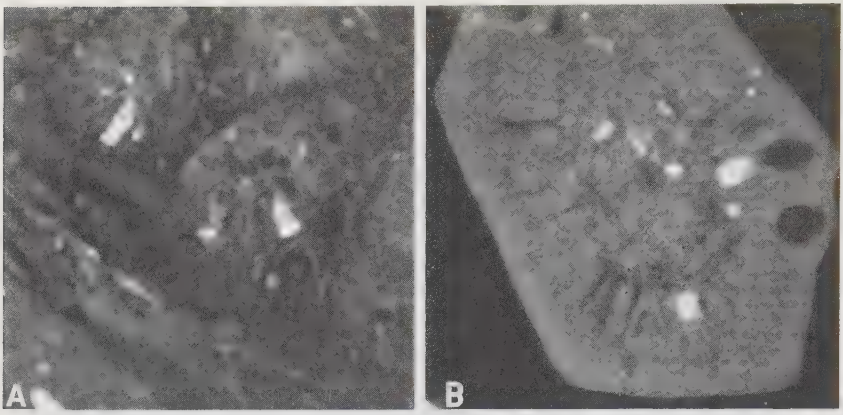


FIG. 3. Immunofluorescent demonstration of endocrine cells in the mouse gut. A, Substance P cells in the duodenum; B, neurotensin cell in th ileum (×400).

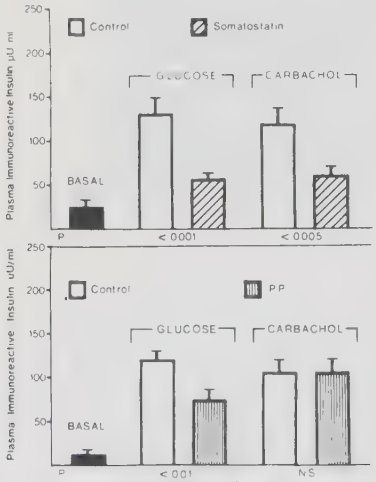


FIG. 4. Plasma immunoreactive insulin in the basal state and peak levels after the iv injection of a half-maximal dose of D-glucose (2.78 mmol/kg) or carbachol (0.16 μmol/kg). Somatostatin or PP (4.25 nmol/kg) was injected iv 15 sec before the secretagogues, respectively. There were 12–16 animals in each group. Bars indicate SEMs. P, Probability level of random difference; NS, not significant.

secretion induced by carbachol. The effects of the gut peptide, substance P, and neurotensin (4.25 nmol/kg) each on stimulated insulin release are shown in Fig. 5. From Fig. 5 it appears that substance P induced a moderate decrease of about 40% on glucose-stimulated insulin release, whereas no effect was observed on the cholinergic insulin response. Neurotensin, on the other hand, had no apparent effect on glucose-induced insulin secretion but enhanced the insulin response to carbachol (Fig. 5).

In order to test whether the observed peptidergic mod ulation of stimulated insulin secretion could be repro duced at larger dose levels, a series of experiments wit a 10-fold higher concentration of the different peptide was performed. Figures 6 and 7 show that the high dos of the different peptides induced a modulation of stimu

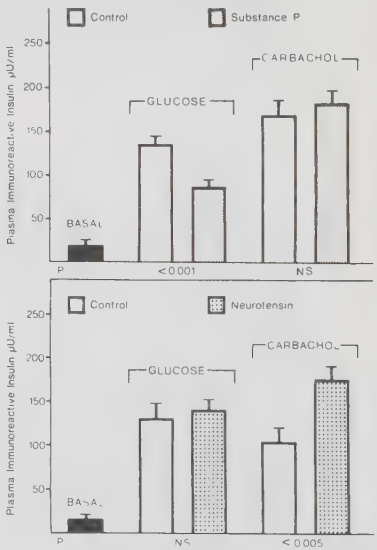


FIG. 5. Plasma immunoreactive insulin in the basal state and pea levels after the iv injection of a half-maximal dose of D-glucose (2.7 mmol/kg) or carbachol (0.16 μmol/kg). Substance P or neurotensi (4.25 nmol/kg) was given iv 15 sec before the secretagogues, respe tively. There were 15–38 animals in each group. Bars indicate SEMs. Probability level of random difference.

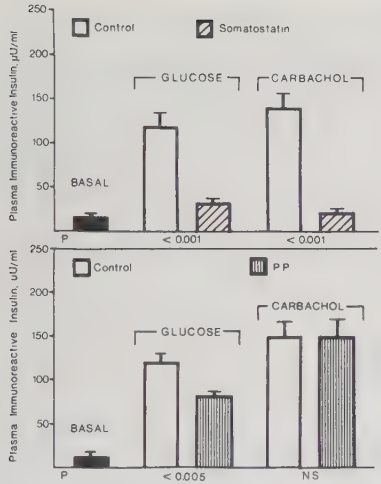


FIG. 6. Plasma immunoreactive insulin in the basal state and peak levels after the iv injection of a half-maximal dose of D-glucose (2.78 mmol/kg) or carbachol (0.16 μmol/kg). Somatostatin or PP (42.5 nmol/kg) was injected iv 15 sec before the secretagogues, respectively. There were 10–28 animals in each group. Bars indicate SEMs. P, Probability level of random difference.

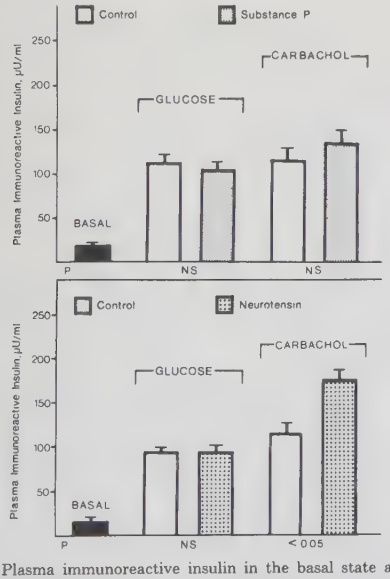


FIG. 7. Plasma immunoreactive insulin in the basal state and peak levels after the iv injection of a half-maximal dose of D-glucose (2.78 mmol/kg) or carbachol (0.16 μmol/kg). Substance P or neurotensin (42.5 nmol/kg) was given iv 15 sec before the secretagogues, respectively. There were 10–35 animals in each group. Bars indicate SEMs. P, Probability level of random difference.

lated insulin secretion similar to that evoked by the lower dose except that substance P had no effect on glucose-induced insulin release. The response to somatostatin showed a definite dose dependence, whereas the modulation exerted by PP and neurotensin was of approximately the same magnitude as with the lower dose.

Discussion

Pancreatic islets of the mouse are composed of at least four different hormone-producing cell types, in accord with recent observations in other species (15, 19, 24). In addition to insulin- and glucagon-containing cells, the mouse islets contain cells storing somatostatin and PP, respectively. The insulin-containing cells make up the central core of the islets, whereas glucagon, somatostatin, and PP cells together form a peripheral zone (Fig. 1). Somatostatin exerts an inhibitory action on insulin secretion induced by various insulin secretagogues, such as glucose, arginine, tolbutamide, theophylline, and the β-adrenergic agonist isoproterenol (25–27), and, as shown here, somatostatin also inhibits insulin release induced by cholinergic stimulation. The dual localization of somatostatin-containing cells, in the pancreatic islets and in the gut, suggests that this peptide may participate in the regulation of insulin secretion, both as a local and a circulating hormone. Its possible implication in the development of diabetes has recently been discussed (28).

The inhibitory effect of somatostatin on the insulin secretory response to glucose and carbachol suggests involvement of somatostatin on a secretory step common to both. Equimolar doses of PP did not affect insulin secretion stimulated by carbachol but caused a moderate inhibition of glucose-induced insulin release. This finding together with the observed distribution of PP-containing cells within the islets suggests a possible role for PP as a local regulator of glucose-induced insulin secretion. Such an assumption might be consistent with recent findings of elevated concentrations of plasma PP in diabetic patients (29) and an increase in PP cell mass in human (30) and experimental (31) diabetes.

Neurotensin and substance P were isolated from bovine hypothalamus by Leeman and coworkers (32–34) and were later found to occur in endocrine cells of the gut also (11, 12, 16, 17). Both peptides induce hypotension, gut contraction, and hyperglycemia (32, 35–38) and have recently been shown to decrease basal plasma insulin levels in rats (37). The present study shows that both neurotensin and substance P occur in endocrine cells of the mouse gut. Substance P was found to have a complex action on glucose-induced insulin secretion; the lower dose gave a moderate inhibition and the higher dose was without effect. Neurotensin in equimolar doses had no apparent effect. On the other hand, neurotensin caused an increase in insulin release induced by car-

bachol, whereas substance P did not appreciably affect cholinergic insulin secretion. These observations suggest that modulation of insulin secretion by these peptides is greatly dependent on the nature of the secretory stimulus.

The present immunohistochemical observations provide a morphological background for the interpretation of the functional roles of somatostatin, PP, neurotensin, and substance P. Since somatostatin and PP occur in the pancreatic islet tissue, they have the potential capability of affecting insulin-secreting cells locally. Both peptides suppressed insulin secretion induced by glucose. Somatostatin was a stronger inhibitor than PP and was also found to substantially inhibit cholinergic insulin release, in contrast to PP, suggesting a different mode of action of the two peptides. Similarly, the gut peptides, substance P and neurotensin, displayed different effects on insulin release stimulated by glucose and carbachol, respectively.

The physiological significance of the polypeptides studied in the regulation of insulin secretion remains unclear. The present observations suggest that caution must be exercised when making functional interpretations, since the actions of these polypeptides might vary with the nature of the insulin secretory stimulus.

Acknowledgments

The expert technical assistance of Mrs. Lena Kvist and Mr. Peter Okmark is gratefully acknowledged.

References

1. Pearse, A. G. E., J. M. Polak, and S. R. Bloom, The newer gut hormones. Cellular sources, physiology, pathology, and clinical aspects, *Gastroenterology* **72:** 746, 1977.
2. Sundler, F., J. Alumets, and R. Håkanson, Peptides in the gut with a dual distribution in nerves and endocrine cells, In Bloom, S. R. (ed.), Gut Hormones, Churchill-Livingstone, London, 1978, pp. 406–412.
3. Hökfelt, T., S. Efendic, C. Hellerström, O. Johansson, R. Luft, and A. Arimura, Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A_1-cells of the pancreatic islets and to the hypothalamus, *Acta Endocrinol (Kbh)* [*Suppl*] **80:** 200, 1975.
4. Dubois, M. P., Presence of immunoreactive somatostatin in discrete cells of the endocrine pancreas, *Proc Natl Acad Sci* **70:** 1340, 1975.
5. Rufener, C., and M. P. Dubois, Cells immunofluorescent to antigrowth hormone release inhibiting factor (somatostatin) in endocrine pancreas and digestive tract, *Diabetologia* **11:** 373, 1975.
6. Arimura, A., H. Sato, A. DuPont, N. Nishi, and A. V. Schally, Somatostatin: abundance of immunoreactive hormone in rat stomach and pancreas, *Science* **189:** 1007, 1975.
7. Pearse, A. G. E., J. M. Polak, S. R. Bloom, C. Adams, J. R. Dryburgh, and J. C. Brown, Enterochromaffin cells of the mammalian small intestine as the source of motilin, *Virchows Arch* [*Zellpathol*] **16:** 111, 1974.
8. Pearse, A. G. E., The cellular origin of motilin in the gastrointestinal tract, *Scand J Gastroenterol* (*Suppl 11*) **39:** 35, 1976.
9. Forssmann, W. G., N. Yanaihara, V. Helmstaedter, and D. Grube, Differential demonstration of the motilin cell and the enterochromaffin cell, *Scand J Gastroenterol* (*Suppl 11*) **39:** 43, 1976.
10. Polak, J. M., S. R. Bloom, M. Kuzio, J. C. Brown, and A. G. E. Pearse, Cellular localization of gastric inhibitory polypeptide in the duodenum and jejunum, *Gut* **14:** 284, 1973.
11. Orci, L., D. Baetens, C. Rufener, M. Brown, W. Vale, and R. Guillemin, Evidence for immunoreactive neurotensin in dog intestinal mucosa, *Life Sci* **19:** 559, 1976.
12. Sundler, F., R. Håkanson, R. A. Hammer, J. Alumets, R. Carraway, S. E. Leeman, and E. Zimmerman, Immunohistochemical localization of neurotensin in endocrine cells of the gut, *Cell Tissue Res* **178:** 313, 1977.
13. Larsson, L.-I., F. Sundler, R. Håkanson, H. G. Pollock, and J. R. Kimmel, Localization of APP, a postulated new hormone, to a pancreatic endocrine cell type, *Histochemistry* **42:** 377, 1974.
14. Larsson, L.-I., F. Sundler, and R. Håkanson, Immunohistochemical localization of human pancreatic polypeptide (HPP) to a population of islet cells, *Cell Tissue Res* **156:** 167, 1975.
15. Larsson, L.-I., F. Sundler, and R. Håkanson, Pancreatic polypeptide—a postulated new hormone: identification of its cellular storage site by light and electron microscopic immunocytochemistry, *Diabetologia* **12:** 211, 1976.
16. Pearse, A. G. E., and J. M. Polak, Immunocytochemical localization of substance P in mammalian intestine, *Histochemistry* **41:** 373, 1975.
17. Nilsson, G., L.-I. Larsson, R. Håkanson, E. Brodin, B. Pernow, and F. Sundler, Localization of substance P-like immunoreactivity in mouse gut, *Histochemistry* **43:** 97, 1975.
18. Larsson, L.-I., J. Fahrenkrug, O. Schaffalitzky de Muckadell, F. Sundler, R. Håkanson, and J. F. Rehfeld, Localization of vasoactive intestinal polypeptide (VIP) to central and peripheral neurons, *Proc Natl Acad Sci* **73:** 3197, 1976.
19. Larsson, L.-I., F. Sundler, and R. Håkanson, Pancreatic hormones in the gut and gut hormones in the pancreas, *In* Fujita, T. (ed.), Endocrine Gut and Pancreas, Elsevier Scientific Publishing Co., Amsterdam, 1976, p. 133.
20. Sundler, F., R. Håkanson, L.-I. Larsson, E. Brodin, and G. Nilsson, Substance P in the gut: an immunochemical and immunohistochemical study of its distribution and development, *In* von Euler, U. S., and B. Pernow (eds.), Substance P, Raven Press, New York, 1977, p. 59.
21. Alumets, J., F. Sundler, and R. Håkanson, Distribution, ontogeny and ultrastructure of somatostatin immunoreactive cells in pancreas and gut, *Cell Tissue Res* **185:** 465, 1977.
22. Rerup, C., and I. Lundquist, Blood glucose level in mice. I. Evaluation of a new technique of multiple serial sampling, *Acta Endocrinol* (*Kbh*) **52:** 357, 1966.
23. Heding, L., A simplified insulin radioimmunoassay method, *In* Donato, L., G. Milhaud, and J. Sirchis (eds.), Labelled Proteins in Tracer Studies, Euratom, Brussels, 1966, p. 345.
24. Orci, L., D. Baetens, M. Ravazzola, Y. Stefan, and F. Malaisse-Lagae, Pancreatic polypeptide and glucagon: non-random distribution in pancreatic islets, *Life Sci* **19:** 1811, 1976.
25. Efendic, S., R. Luft, and V. Grill, Effect of somatostatin on glucose induced insulin release in isolated perfused rat pancreas and isolated rat pancreatic islets, *FEBS Lett* **42:** 169, 1974.
26. Gerich, J. E., R. Lovinger, and G. M. Grodsky, Inhibition by somatostatin of glucagon and insulin release from the perfused rat pancreas in response to arginine, isoproterenol and theophylline: evidence for a preferential effect on glucagon secretion, *Endocrinology* **96:** 749, 1975.
27. Fujimoto, W. Y., Somatostatin inhibition of glucose-, tolbutamide-, theophylline-, cytochalasin B-, and calcium-stimulated insulin release in monolayer cultures of rat endocrine pancreas, *Endocrinology* **97:** 1494, 1975.
28. Orci, L., D. Baetens, C. Rufener, M. Amherdt, M. Ravazzola, P. Studer, F. Malaisse-Lagae, and R. H. Unger, Hypertrophy and hyperplasia of somatostatin-containing D-cells in diabetes, *Proc Natl Acad Sci* **73:** 1338, 1976.
29. Floyd, Jr., J. C., S. S. Fajans, S. Pek, and R. E. Chance, A newly recognized pancreatic polypeptide; plasma levels in health and disease, *Recent Prog Horm Res* **33:** 519, 1977.
30. Gepts, W., J. De Mey, and M. Marichal-Pipeleers, Hyperplasia of "pancreatic polypeptide"-cells in the pancreas of juvenile diabetes, *Diabetologia* **13:** 27, 1977.
31. Sundler, F., R. Håkanson, I. Lundquist, and L.-I. Larsson, Effect of alloxan on rat pancreatic polypeptide (PP) cells, *Cell Tissue Res* **178:** 307, 1977.
32. Carraway, R., and S. E. Leeman, The isolation of a new hypotensive

peptide, neurotensin, from bovine hypothalami, *J Biol Chem* **248:** 6854, 1973.
133. Carraway, R., and S. E. Leeman, The amino acid sequence of a hypothalamic peptide, neurotensin, *J Biol Chem* **250:** 1907, 1975.
134. Chang, M. M., and S. W. Leeman, Isolation of a sialogenic peptide from bovine hypothalamic tissue and its characterization as substance P, *J Biol Chem* **245:** 4784, 1970.
135. Leeman, S. E., and E. A. Mroz, Minireview: substance P, *Life Sci* **15:** 2033, 1975.
36. Carraway, R. E., L. M. Demers, and S. E. Leeman, Hyperglycemic effect of neurotensin, a hypothalamic peptide, *Endocrinology* **99:** 1452, 1976.
37. Brown, M., and W. Vale, Effects of neurotensin and substance P on plasma insulin, glucagon and glucose levels, *Endocrinology* **98:** 819, 1976.
38. Lembeck, F., and G. Zetler, A polypeptide of possible physiological significance, especially within the nervous system, *Int Rev Neurobiol* **4:** 159, 1962.

Adrenergic Innervation of Pancreatic Islets and Modulation of Insulin Secretion by the Sympatho-Adrenal System

Bo Ahrén, Lars E. Ericson, Ingmar Lundquist, Ingemar Lorén and Frank Sundler

Departments of Pharmacology and Histology, University of Lund and Department of Anatomy, University of Gothenburg, Sweden

SUMMARY

Morphological changes in the adrenergic innervation of pancreatic islets after chemical sympathectomy by 6-hydroxydopamine, and the influence of the sympatho-adrenal system on insulin secretion were investigated in the mouse and rat.

Fluorescence histochemistry revealed a clear-cut reduction in the number of adrenergic nerve fibers in the pancreatic islets 2 days after administration of 6-hydroxydopamine; the reduction was more pronounced in the rat than in the mouse. In the rat, a partial regeneration was seen after 6 weeks.

In the pancreas of the mouse, a severe damage of unmyelinated nerve fibers after administration of 6-hydroxydopamine was revealed electron microscopically. No ultrastructural or immunohistochemical alterations could be demonstrated in the islet endocrine cells.

6-Hydroxydopamine induced a depression of basal plasma insulin concentrations in mice and an elevation in rats. Adrenalectomy depressed basal plasma insulin levels in mice.

The α-adrenoceptor antagonist phentolamine enhanced insulin secretion in normal mice. The insulin secretory response to phentolamine was diminished by chemical sympathectomy and almost abolished by adrenalectomy or the combination of chemical sympathectomy and adrenalectomy. Thus, the effect of phentolamine is probably mediated by liberated catecholamines.

It is concluded that basal insulin secretion is partially regulated by the sympatho-adrenal system and that species differences exist in this respect. In addition, the results suggest that endogenous catecholamines have the ability to promote insulin secretion.

INTRODUCTION

It is a long held view that insulin secretion is modulated by the sympatho-adrenal system, which encompasses adrenergic nerves as well as hormones (noradrenaline and adrenaline) secreted by the adrenal medulla (cf. Woods and Porte, 1974; Gerich and Lorenzi, 1978).

Fluorescence microscopy has revealed a rich supply of catecholaminergic nerve fibers in the pancreas of a variety of mammalian species (Falck and

Hellman, 1964; Cegrell, 1968) and electron microscopic autoradiography has demonstrated such nerve fibers in close connection to the different types of islet cells (Lundquist and Ericson, 1978). These findings suggest that islet adrenergic fibers might be of importance for the regulation of pancreatic hormone release, and electrical stimulation of the adrenergic nerves as well as activation of the sympatho-adrenal system have been shown to result in inhibition of insulin release (Schalch, 1967; Wright and Malaisse, 1968; Bloom and Edwards, 1975; Järhult and Holst, 1979). Furthermore, noradrenaline and adrenaline both inhibit the secretion of insulin when administered in vivo (Porte, 1967; Porte and Williams, 1966). However, a concomitant administration of the α-adrenergic blocker phentolamine reverses this inhibition to a stimulation (Porte, 1967; Lundquist, 1972b). Thus it has been proposed that the sympatho-adrenal system exerts inhibitory as well as stimulatory effects on the insulin cells; effects mediated by inhibitory α-adrenergic receptors and by stimulatory β-adrenergic receptors (cf. Lundquist, 1971; Woods and Porte, 1974).

The aim of the present investigation was to study in more detail the role of the sympatho-adrenal system for basal insulin secretion. In addition, the occurrence and distribution in the pancreas of adrenergic nerves as well as their ultrastructure were studied before and after 6-hydroxydopamine administration.

MATERIAL AND METHODS

Animals. Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25-35 g, and female rats of the Wistar strain (Møllegaard Avlslaboratorium, Skensved, Denmark), weighing 250-300 g, were used throughout the experiments. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum before and during the experiments. Adrenalectomy was performed by the lumbar approach under light diethylether anaesthesia. Following adrenalectomy the mice were maintained on the usual diet and given 0.9% NaCl to drink ad libitum.

Fluorescence Histochemistry of Adrenergic Nerves. 6-Hydroxydopamine, dissolved in 0.9% NaCl and ascorbic acid, 10 mg/ml, was given to mice (40 mg/kg) and rats (65 mg/kg). Controls were given the vehicle. Two days or six weeks later the animals were anaesthetized by i.p. injection of pentobarbitone and perfused via the heart in two steps for fixation. The animals were first perfused with 50 ml (mouse) or 150 ml (rat) of a Tyrode buffer and then with 100 ml (mouse) or 300 ml (rat) of a Tyrode buffer with 30 g $Al_2(SO_4)_3 \times 18$ H_2O and 2% paraformaldehyde added. pH was adjusted to 3.8 by means of 1.14 g $Na_2B_4O_2 \times 10$ H_2O/300 ml. For further details of the composition of the perfusion solutions and the perfusion technique see Ajelis et al. 1979; Lorén et al. 1980a and b. Pieces of the pancreas were rapidly frozen in a mixture of propane and propylene, cooled to the temperature of liquid nitrogen, and freeze-dried. The freeze-dried tissue pieces were treated with formaldehyde vapour, generated from paraformaldehyde equilibrated in 50% relative humidity, at +80°C for 1 h. The tissue specimens were then embedded in paraffin in vacuo, sectioned at 6 µm and mounted in Entellan (for further details on these procedures, see Björklund et al. 1972).

Immunohistochemistry of Islet Endocrine Cells. Specimens were collected from the splenic portion of the pancreas of rats and mice, before, 2 days and 6 weeks after administration of 6-hydroxydopamine, respectively. The specimens were frozen to the temperature of liquid nitrogen in a mixture of propane and propylene. After freeze-drying, the specimens were fixed with formaldehyde vapour for 1 h at 80°C and embedded in paraffin. Paraffin sections were

cut at 5 µm. After removal of the embedding medium, the sections were processed for the immunohistochemical demonstration of insulin, glucagon, somatostatin and pancreatic polypeptide (PP) using the indirect immunofluorescence procedure of Coons et al. (1955). Details on the immunohistochemical procedures have been reported previously (Larsson et al., 1976). As controls served sections incubated with antiserum inactivated by the addition of antigen in excess (100 µg/ml diluted antiserum). Guinea pig antiserum against pure porcine insulin was a gift from Dr. L.G. Heding, Novo, Copenhagen, Denmark. It was used at a dilution of 1:160. Rabbit antiserum against pure porcine glucagon (code no 4304) was generously donated by Dr. J.J. Holst, Bispebjerg Hospital, Copenhagen, Denmark. It was used at a dilution of 1:80. Antiserum against synthetic somatostatin (code no 19578) was supplied by Dr. M.P. Dubois, Nouzilly, France and used at a dilution of 1:160. Antiserum against pure bovine pancreatic polypeptide was given to us by Dr. R.E. Chance, Eli Lilly Co, Indianapolis, Indiana, USA. The PP antiserum was used at a dilution of 1:640. The antisera have been used in previous immunocytochemical studies (see e.g. Lundquist et al., 1979). Fluorescein isothiocyanate-labelled goat antiserum against rabbit IgG or against guinea pig IgG was purchased from Statens Bakteriologiska Laboratorium, Stockholm, Sweden. Synthetic somatostatin was from Beckman Ltd, Geneva, Switzerland, pure porcine insulin and glucagon were gifts from Dr. L.G. Heding, Novo, Copenhagen, Denmark and pure bovine PP was a gift from Dr. R.E. Chance, Eli Lilly Co, Indianapolis, Indiana, USA.

Electron Microscopy. Mice (4 in each group) were injected i.v. with 6-hydroxydopamine dissolved in 0.9% NaCl containing ascorbic acid, 10 mg/ml; 40 or 80 mg/kg were given. Controls received the vehicle alone. After 48 h the pancreas was fixed by perfusion via the left heart ventricle with 3% glutaraldehyde in 0.05 M sodium cacodylate. After excision, pieces of the gland were further immersed in glutaraldehyde for 1-2 h and then postfixed in 1% osmium tetroxide for 1 h. Specimens were embedded in Epon and sections stained with uranyl acetate and lead citrate.

Experiments. The effect on insulin secretion of manipulations with the sympatho-adrenal system was studied in mice and rats and of α-adrenergic blockade by phentolamine was investigated in mice. Mice were subjected to adrenalectomy and/or chemical sympathectomy by 6-hydroxydopamine. Adrenalectomized mice were treated with 6-hydroxydopamine 8-14 days after the operation. 6-Hydroxydopamine was dissolved in 0.9% NaCl containing ascorbic acid, 10 mg/ml, and injected i.v. in a tail vein; 40 mg/kg was given. Controls were given the vehicle. After another 2-8 days, phentolamine was given i.p., 0.13 mmol/kg body weight, immediately after a 0 min blood sample had been taken; blood samples were taken at different time intervals thereafter. In control animals saline was injected. In another series of experiments 6-hydroxydopamine, dissolved in 0.9% NaCl containing ascorbic acid, 10 mg/ml, was injected i.v. to mice (40 mg/kg) and rats (65 mg/kg) and blood samples were taken at different time intervals thereafter. Blood was sampled by orbital puncture (Rerup and Lundquist, 1966). Phentolamine methanesulfonate was supplied by CIBA-Geigy Ltd, Basel, Switzerland, 6-hydroxydopamine from Hässle AB, Mölndal, Sweden and ascorbic acid from E. Merck AG, Darmstadt, BRD.

Determination of Insulin and Glucose. The concentration of immunoreactive insulin (IRI) in plasma was determined by radioimmunoassay (Heding, 1966), using ^{125}I-labelled pig insulin and guinea pig anti-pig insulin. Blood and plasma glucose concentration was determined enzymatically (Bruss and Black, 1978).

Statistics. Means and SEMs are shown. For statistical analysis Student's paired and non-paired t-test were employed.

RESULTS

1. Fluorescence Histochemistry of Adrenergic Nerves

Controls. Numerous delicate varicose green-fluorescent adrenergic nerve fibers were seen in the islets of Langerhans in mice (Fig. 1a and b) and rats (Fig. 2). The nerve fibers appeared to enter the islets via small blood vessels. Most nerve fibers were located in the periphery of the islets. In the exocrine pancreas, scattered thin non-varicose as well as varicose fluorescent fibers occurred among the acini (Figs 1c and 2). Blood vessels of varying size were often richly innervated by adrenergic nerve fibers. Generally the rat harboured slightly fewer adrenergic nerves in the pancreas than the mouse.

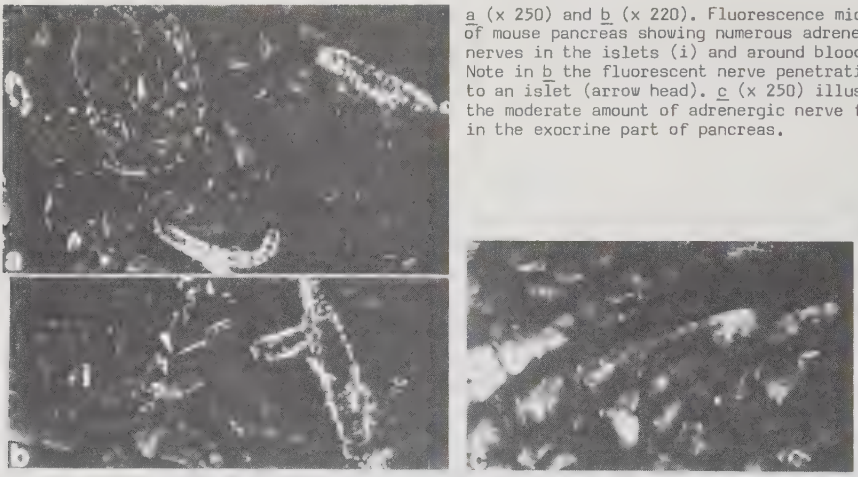


Fig. 1

a (x 250) and b (x 220). Fluorescence micrographs of mouse pancreas showing numerous adrenergic nerves in the islets (i) and around blood vessels Note in b the fluorescent nerve penetrating into an islet (arrow head). c (x 250) illustrates the moderate amount of adrenergic nerve fibers in the exocrine part of pancreas.

Fig. 2

Fluorescence micrograph of rat pancreas demonstrating the innervation of the endocrine (i) as well as exocrine part (x 250).

6-Hydroxydopamine-Treated Mouse. At 2 days after 6-hydroxydopamine there was a clear-cut reduction of adrenergic nerve fibers in some regions of the pancreas (Fig. 3a). The strongest effect was seen on the fibers in the wall of larger blood vessels while those in the wall of smaller vessels were less affected. In certain regions the adrenergic nerves in both the exocrine and endocrine pancreas were almost totally lost. In other regions of the same tissue section the innervation pattern was roughly the same as that of control animals. Six weeks after 6-hydroxydopamine there was a moderate decrease of nerves in some islets, while other islets were totally devoid of nerves (Fig. 4a); thus the fluorescent microscopical picture of the islets was almost identical to that seen two days after 6-hydroxydopamine (cf. Fig. 3a). Further, after 6 weeks an almost total loss of adrenergic nerves in the exocrine part of pancreas (Fig. 4b) and a marked, though not total, reduction of the adrenergic innervation of the vascular bed were seen.

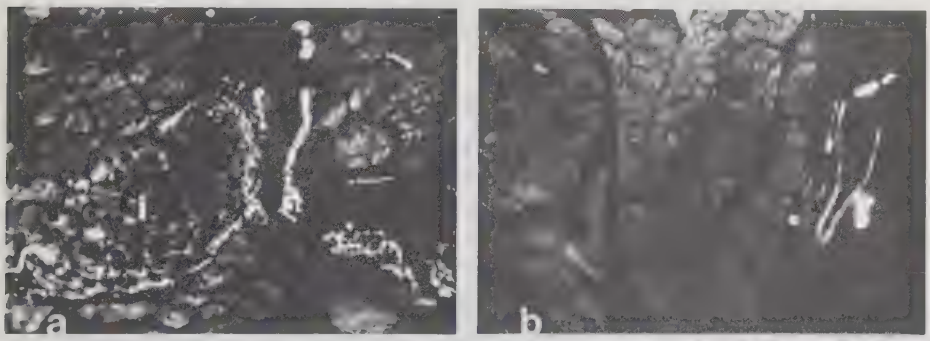


Fig. 3

Fluorescence micrograph of the mouse (a) (x 280) and rat (b) (x 260) pancreas 2 days after administration of 6-hydroxydopamine. Note in the mouse (a) some adrenergic nerve fibers in the islet (I) periphery as well as around blood vessels persisted, whereas in the rat (b) adrenergic nerve fibers were absent except around large blood vessels.

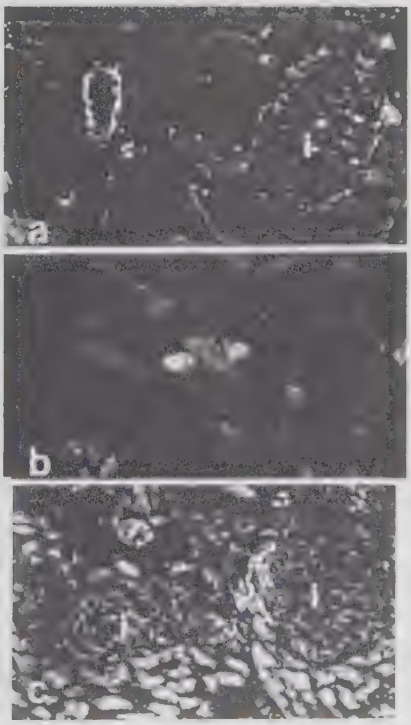


Fig. 4

Fluorescence micrograph of the mouse endocrine (a) (x 280) and exocrine (b) (x 300) and rat (c) (x 250) pancreas 6 weeks after administration of 6-hydroxydopamine. In the mouse, adrenergic nerve fibers are still reduced in number. (cf. Figs 1 and 3). In the rat, there is a partial regeneration of the adrenergic innervation from the almost total loss seen 2 days after 6-hydroxydopamine (cf. Fig. 3) both in endocrine (i) and exocrine pancreas.

6-Hydroxydopamine Treated Rat. At 2 days after 6-hydroxydopamine there was an almost total loss of adrenergic nerve fibers both in the pancreatic islets and in the exocrine parenchyma. Only along large blood vessels were bundles of coarse adrenergic fibers seen (Fig. 3b). Six weeks after 6-hydroxydopamine adrenergic nerve fibers had reappeared (Fig. 4c) although the distri- of adrenergic nerves was patchy. In some areas the number and distribution of adrenergic nerves was similar to that seen in the control animals whereas in other areas the number of such fibers was reduced in both the exocrine and endocrine parenchyma as well as in the wall of blood vessels. The greatest reduction of nerves was seen in the wall of the larger vessels.

2. Immunohistochemistry of Islet Endocrine Cells. In the control mice, numerous insulin cells occupied the central region of the islets. Glucagon cells and PP cells occupied the peripheral parts and somatostatin cells occurred intermingled with other islet cells mostly in the peripheral zone. Glucagon cells occurred in a moderate number whereas PP cells and somatostatin cells were few. There were no changes in the number and distribution of the islet cells after 6-hydroxydopamine administration, neither 2 days (Fig. 5) nor 6 weeks after treatment. The cellular content of the different hormones was not visibly altered after 6-hydroxydopamine.

Identical findings as in mice were made in rats, both with regard to frequency distribution of the different islet cells in controls as well as the lack of detectable changes after 6-hydroxydopamine (not shown in Fig.).

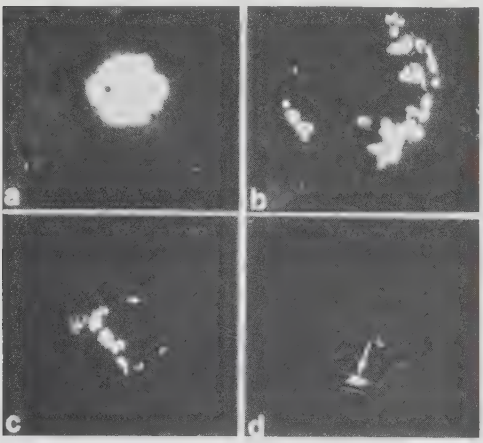


Fig. 5

Mouse pancreas 2 days after administration of 6-hydroxydopamine. Sections through islets showing the immunohistochemical distribution of insulin cells (a), glucagon cells (b), somatostatin cells (c), and PP cells (d). The single PP cell demonstrated in d is marked by arrow. The number or distribution of the different islet cells does not differ from controls. (x 200).

3. Electron Microscopy. Administration of 6-hydroxydopamine induced morphological changes in unmyelinated nerves in the pancreas (Figs. 6b-e). These changes were qualitatively and quantitatively similar after both doses of 6-hydroxydopamine (40 mg/kg and 80 mg/kg). Many terminals were filled with necrotic, electron-dense material (Figs. 6b and c). Other terminals were less severely affected and were swollen with a reduced content of synaptic vesicles (Figs. 6d and e). Affected terminals were most frequently found in relation to smooth muscle cells in the wall of blood vessel but they were also present close to exocrine cells and in the interstitial connective tissue (Figs. 6b and c). Ultrastructurally changed terminals were also found in relation to different types of islet cells, including insulin cells (Figs. 6d and e). Such nerves were most often located in the islet periphery.

6-Hydroxydopamine did not induce any apparent alterations in the ultrastructure of any of the different types of islet endocrine cells. Thus, the insulin-producing cells were well granulated and did not display any signs of degeneration (Fig. 6a).

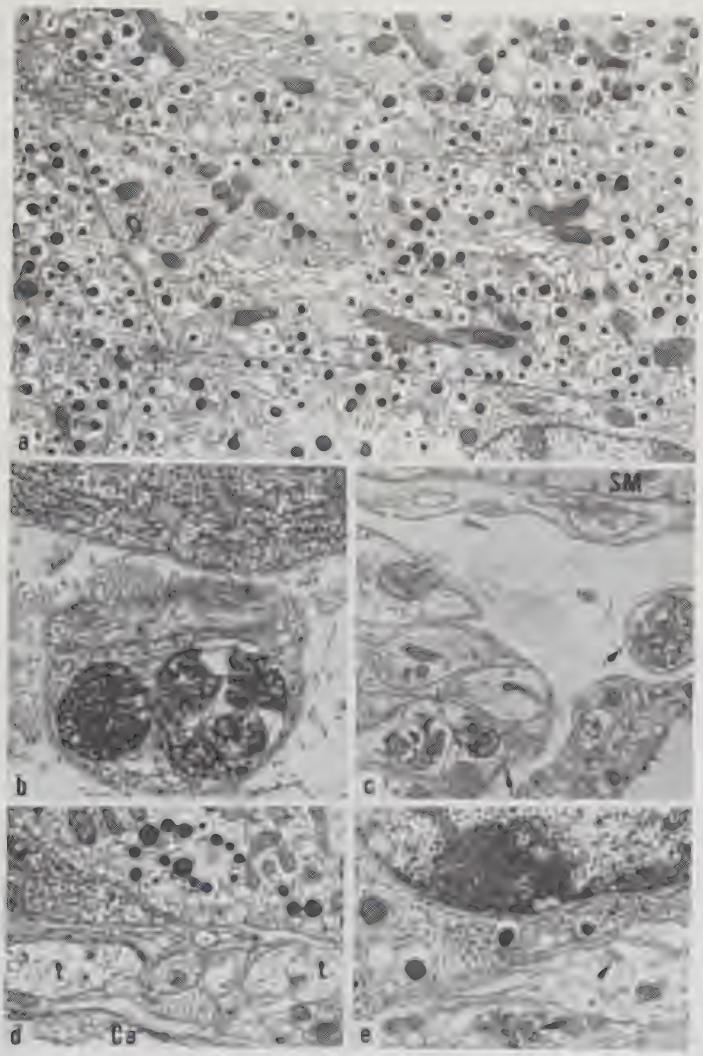


Fig. 6. Electron micrographs of mouse pancreas 2 days after injecting of 6-hydroxydopamine. a. Portions of 4 islet insulin cells. The ultrastructure is unaffected. (x 8500) b. Two adrenergic nerve terminals, located close to an exocrine cells, filled with dense, necrotic material. (x 17 500). c. Unmyelinated nerves located in connective tissue close to a smooth muscle cell (SM) located in the wall of an arteriole. Two of the nerves (arrows) contain accumulations of necrotic material. (x 10 000). d. Nerves located in the periphery of an islet and close to a capillary (Ca). Two terminals (t) contain very few synaptic vesicles. (x 10 000). e. Nerve terminals located close to an insulin cell. One terminal appears to be swollen and contains only a few synaptic vesicles (arrow head). Another terminal contains dense material (arrow). (x 17 500).

4. Basal Insulin Secretion

Chemical sympathectomy was induced by injection of 6-hydroxydopamine in mice (40 mg/kg) and rats (65 mg/kg). Fig. 7 shows the plasma insulin and glucose concentrations during 45 days thereafter. In the mouse, mean plasma insulin concentrations had a lower value in 6 of the 7 blood sampling periods after chemical sympathectomy than in controls; the mean difference between 6-hydroxydopamine treated and control mice over the sampling period was -10.0 ± 3.6 μU/ml ($p < 0.05$). In the rat, mean plasma insulin concentrations had a higher value in all 7 blood sampling periods after chemical sympathectomy than in controls; the mean difference between 6-hydroxydopamine treated and control rats over the sampling period was 15.5 ± 4.2 μU/ml ($p < 0.05$). Thus, a species difference existed between mice and rats in this respect ($p < 0.001$).

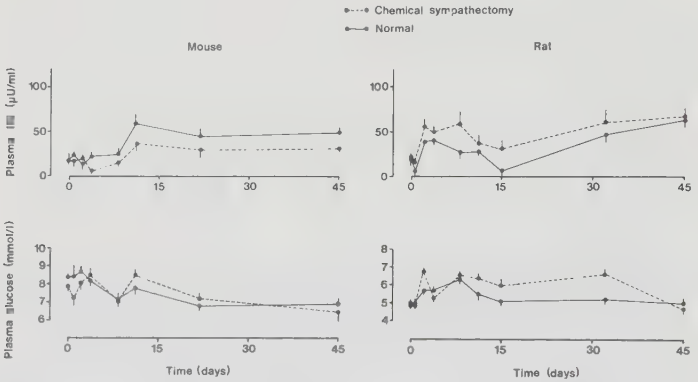


Fig. 7. Plasma concentrations of immunoreactive insulin (upper panel) and glucose (lower panel) in mice and rats subjected to chemical sympathectomy compared with normal animals. Chemical sympathectomy was induced by 6-hydroxydopamine immediately after the first sample had been taken. Bars indicate SEMs. There were 6 animals in each group.

Plasma glucose appeared to be slightly elevated 2-4 weeks after injection of 6-hydroxydopamine in rats, whereas in mice no appreciable changes were noted with regard to plasma glucose.

In the mouse, adrenalectomy or chemical sympathectomy depressed basal plasma insulin levels by approximately 20% ($p < 0.02$). Blood glucose concentrations were marginally depressed (by about 10%, $p < 0.02$) in the two groups of mice (Fig. 8). The combined treatment of adrenalectomy and chemical sympathectomy depressed basal plasma insulin markedly, by about 40% ($p < 0.001$), and also blood glucose was lowered, by about 20% ($p < 0.001$) (Fig. 8).

5. Effects of α-Adrenergic Blockade

The α-adrenoceptor antagonist phentolamine induced an enhanced rate of insulin secretion. It elevated plasma insulin concentrations from 50 ± 9 μU/ml to 86 ± 15 μU/ml ($p < 0.05$) at 6 min and to 82 ± 11 μU/ml ($p < 0.05$) at 10 min after injection (Fig. 9). Plasma glucose concentrations were depressed after exposure to phentolamine; the value 15 min after injection

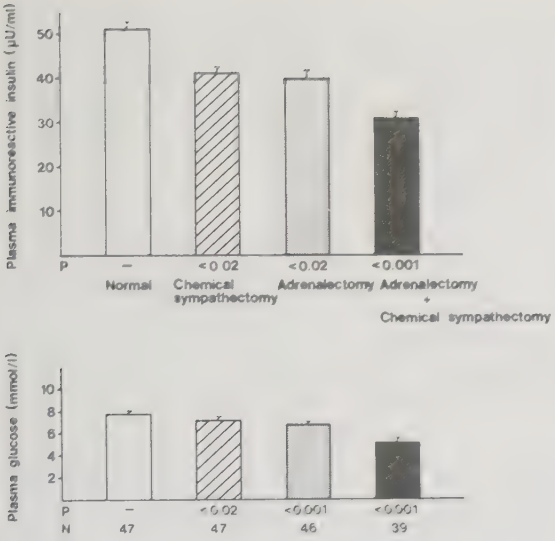


Fig. 8

Plasma concentrations of immunoreactive insulin (upper panel) and glucose (lower panel) in normal mice, chemically sympathectomized mice, adrenalectomized mice and in mice subjected to combination of chemical sympathectomy and adrenalectomy. Adrenalectomy was performed 16-22 days before the blood sampling and chemical sympathectomy by 6-hydroxydopamine 2-8 days before the sampling of blood. Control animals were injected 2-8 days before sampling. Bars indicate SEMs. N number of animals. P probability level of random difference versus normal mice.

was 6.5 ± 0.3 mmol/l compared to 9.7 ± 0.2 mmol/l in controls ($p < 0.001$) (Fig. 9). Since peak response in plasma insulin concentrations occurred 6-10 min after phentolamine administration, blood was sampled immediately before and 10 min after phentolamine administration in subsequent experiments.

In the next series of experiments the insulin secretory response to phentolamine was investigated in normal mice and in mice subjected to adrenalectomy and/or chemical sympathectomy by 6-hydroxydopamine. In normal mice, phentolamine induced a marked elevation of plasma insulin concentrations, from 18 ± 2 μU/ml to 61 ± 7 μU/ml ($p<0.001$) (Fig. 10). In chemically sympathectomized mice, phentolamine, likewise, elevated plasma insulin levels ($p < 0.01$), but

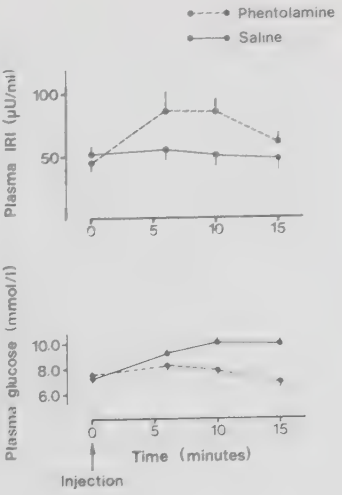


Fig. 9

Plasma immunoreactive insulin (upper panel) and glucose (lower panel) in the basal state and 6, 10 and 15 min after the i.p. injection of either phentolamine, 0.13 mmol/kg, or saline in normal mice. Bars indicate SEMs. There were 15 animals in each group.

the elevation was less pronounced than in normal mice. In adrenalectomized mice and in mice subjected to combined chemical sympathectomy and adrenalectomy, α-adrenergic blockade by phentolamine did not induce any appreciable changes in plasma insulin concentrations (Fig. 10). Blood glucose concentrations were depressed in response to phentolamine in normal, adrenalectomized and adrenalectomized-chemically sympathectomized mice, whereas mice subjected to chemical sympathectomy showed no change in blood glucose after phentolamine.

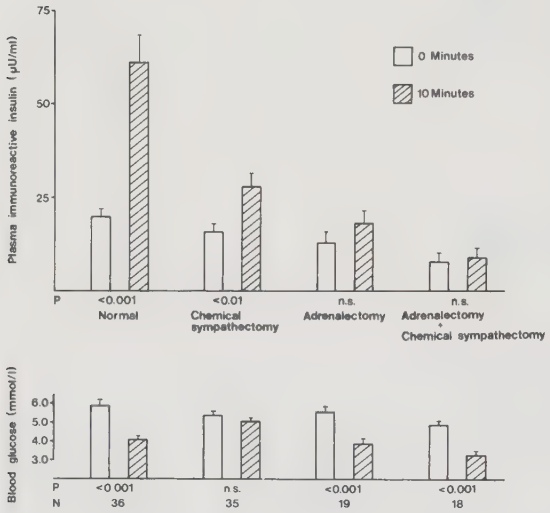


Fig. 10

Plasma concentrations of immunoreactive insulin (upper panel) and blood concentrations of glucose (lower panel) in the basal state and 10 min following the i.p. injection of phentolamine, 0.13 mmol/kg, in normal mice, chemically sympathectomized mice or adrenalectomized mice and in mice subjected to combination of chemical sympathectomy and adrenalectomy. Adrenalectomy was performed 16-22 days before the blood sampling and chemical sympathectomy by 6-hydroxydopamine 2-8 days before the sampling of blood. Control animals were injected with saline 2-8 days before sampling. Bars indicate SEMs. N number of animals. P probability level of random difference. NS not significant.

DISCUSSION

The results of the present study support the view that the adrenergic nerves are of importance for basal insulin secretion in the intact mouse and rat (cf. Woods and Porte, 1974), as deduced from the results obtained in animals treated with 6-hydroxydopamine. This drug is known to selectively accumulate in adrenergic nerve terminals. This leads to a depletion of the catecholamine stores and, eventually, to a destruction of the nerve terminals (Kostrzewa and Jacobowitz, 1974). In the present investigation fluorescence microscopy revealed a clearcut reduction in the number of adrenergic nerve fibers in the endocrine pancreas after administration of 6-hydroxydopamine. Thus, in the rat, 6-hydroxydopamine induced an almost total loss of adrenergic fibers in the islets after 2 days. After 6 weeks some regeneration had occurred. In the mouse, there was a clear-cut reduction of adrenergic fibers in some islets 2 days after 6-hydroxydopamine administration, whereas in other islets the adrenergic innervation pattern was virtually unaffected. This patchy reduction remained also after 6 weeks. In the mouse, electron microscopy demonstrated severe degenerative changes in nerve fibers of the islets, including fibers closely related to insulin cells, 2 days after 6-hydroxydopamine. No ultrastructurally or immunohistochemically demonstrable alterations were noted in the islet endocrine cells after 6-hydroxydopamine.

Mice treated with 6-hydroxydopamine had slightly lower basal insulin concentrations than intact animals. Since the endocrine cells seemed unaltered after 6-hydroxydopamine administration, whereas the adrenergic nerves were

damaged, it is reasonable to assume that the noted changes in insulin secretion after 6-hydroxydopamine largely are due to disturbed adrenergic innervation of the islets. Furthermore, adrenalectomy depressed basal insulin levels in mice and the lowest basal plasma insulin concentration was found after combined 6-hydroxydopamine treatment and adrenalectomy. This indicates the importance of both the sympathetic nerves and the adrenals in maintaining a normal basal insulin concentration. It may be noted that level of circulating glucose was in some animals normal (Fig. 7) and in others marginally depressed (Fig. 8) after adrenalectomy or chemical sympathectomy. Although this might occasionally contribute to the lowered plasma insulin concentrations it was presumably too small to have any major influences.

Earlier the ß-adrenoceptor blocker propranolol has been found to depress basal plasma insulin levels in man (Porte, 1967; Gagliardino et al., 1970), baboon (Werrbach et al., 1970), and mouse (Lundquist 1972a; Lundquist and Ericson, 1978) and splanchnicotomy in the dog has been shown to slightly depress plasma insulin in the basal state (Girardier et al., 1978). Thus, sympatho-adrenal influences seem to modulate basal insulin secretion in several species.

Contrary to the findings in mice, basal plasma insulin concentrations were slightly increased in rats treated with 6-hydroxydopamine. This is in accordance with a previous study (Burr et al., 1974). Further, surgical splanchnicotomy and adrenalectomy enhance plasma insulin in rats (Ahrén, Järhult and Lundquist, 1981). The relative balance between the degree of α- and ß--stimulation may be of importance in maintaining basal insulin and the noted species differences may at least partly be explained by different sensitivity of the insulin cell to α- and ß-adrenergic stimulation in different species (cf. Loubatières-Mariani et al., 1977).

It has recently been proposed that the α-adrenergic receptors can be subdivided into two groups (Langer, 1974; Langer, 1977; Guicheney and Meyer, 1979). The α_1-adrenergic receptors are mainly situated at target cells whereas α_2-adrenergic receptors are presynaptically located, eliciting auto-feedback inhibition of the release of catecholamines from nerve terminals. Since phentolamine blocks both α_1- and α_2-receptors it might induce α_1-blockade concomitantly with a blocked inhibition of release of catecholamines. If the catecholamines by this mechanism are released by phentolamine (cf. Dairman and Udenfriend, 1970), administration of this drug can be regarded as a model of studying the ß-adrenergic component of adrenergic responses to endogenous catecholamines in vivo. Since exogenously induced ß-adrenoceptor stimulation is known to enhance insulin secretion (Lundquist and Ericson, 1978), phentolamine elevates plasma insulin concentrations. This has been found earlier in several species, such as in baboons (Werrbach et al., 1970), sheep (Hertelendy et al., 1970), dogs (Smith, Woods and Porte, 1976), rats (Järhult, Ahrén and Lundquist, 1979), mice (Lundquist, 1972b) and man (Porte, 1967).

Mice treated with 6-hydroxydopamine had a much weaker increase in plasma insulin following phentolamine administration than normal mice, which probably reflects the reduced catecholamine stores in these animals (cf. Fredholm, Farnebo and Hamberger, 1978). Further, the insulin secretory response to phentolamine was almost abolished after the combined treatment of chemical sympathectomy and adrenalectomy. Thus, phentolamine seems to be devoid of any intrinsic insulin releasing action, rather, the effects may be ascribed to those exerted by liberated endogenous catecholamines.

Since phentolamine did not increase insulin release in adrenalectomized mice catecholamines of neural origin seem to be of lesser importance for the ß-adrenergic response. However, an important role of these amines cannot be excluded since the sympathectomized mice had a lower insulin secre-

tion after phentolamine than intact.

In conclusion, this study shows that administration of 6-hydroxydopamine is followed by a substantial reduction in the number of adrenergic nerves in the endocrine and exocrine parts of the pancreas. The islet endocrine cells were ultrastructurally and immunohistochemically unaltered after 6-hydroxydopamine. Further, the results suggest that endogenous catecholamines have an ability to stimulate insulin secretion and that the sympatho-adrenal system is of physiological importance for maintaining basal plasma insulin levels.

ACKNOWLEDGEMENTS

The technical assistance of Ulla Jarl, Lena Kvist, Marianne Maxe-Colschen and Peter Okmark is gratefully acknowledged. The study was supported by grants from Nordisk Insulinfond, Gentofte, Denmark, Svenska Diabetesförbundet, and from the Swedish Medical Research Council (14P-4289, 04X-4289 and 12X-537).

REFERENCES

Ahrén B, Järhult J, Lundquist I (1981) Influence of the sympatho-adrenal system and somatostatin on the secretion of insulin in the rat. J Physiol in press

Ajelis V, Björklund A, Falck B, Lindvall O, Lorén I, Walles B (1979) Application of the aluminium-formaldehyde (ALFA) histofluorescence method for demonstration of peripheral stores of catecholamines and indolamines in freeze-dried paraffin-embedded tissue, cryostat sections and whole-mounts. Histochemistry 65:1-15.

Björklund A, Falck B, Owman Ch (1972) Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In: Berson SA (ed) Methods of investigative and diagnostic endocrinology Vol. I: Rall JF, Kopin IJ (eds) The thyroid and biogenic amines. North-Holland Publ. Co., Amsterdam, pp 318-368

Bloom SR, Edwards AV (1975) The release of pancreatic glucagon and inhibition of insulin in response to stimulation of the sympathetic innervation. J Physiol 253: 157-173

Bruss ML, Black AL (1978) Enzymatic microdetermination of glycogen. Anal Biochem 84: 309-312

Burr IM, Jackson A, Culbert S, Sharp R, Felts P, Olson W (1974) Glucose intolerance and impaired insulin release following 6-hydroxydopamine administration to intact rats. Endocrinology 94: 1072-1076

Cegrell L (1968) The occurrence of biogenic monoamines in the mammalian endocrine pancreas. Acta Physiol Scand Suppl 314: 5-60

Coons AH, Leduc EM, Connolly JM (1955) Studies on antibody production. I. A method for the histochemical demonstration of specific antibodies and its application to a study of the hyperimmune rabbit. J Exp Med 102:49-59.

Dairman W, Udenfriend S (1970) Effect of ganglionic blocking agents on the increased synthesis of catecholamines resulting from α-adrenergic blockade or exposure to cold. Biochem Pharmacol 19:979-984.

Falck B, Hellman B (1964) A fluorescent reaction for monoamines in the insulin producing cells of the guinea-pig. Acta Endocr (Kbh) 45: 133-138

Fredholm BB, Farnebo LO, Hamberger B (1978) Plasma catecholamines, cyclic AMP, and metabolic substrates in hemorrhagic shock of the rat. The effect of adrenal demedullation and 6-OH-dopamine treatment. Acta Physiol Scand 105: 481-495

Gagliardino JJ, Bellone CF, Doria I, Sánchez JH, Pereyra V (1970) Adrenergic regulation of basal serum glucose, NEFA and insulin levels. Horm Metab Res 2: 318-322

Gerich JE, Lorenzi M (1978) The role of the autonomic nervous system and somatostatin in the control of insulin and glucagon secretion. In: Ganong WF, Martini L (eds) Front Neuroendocr, vol 5, Raven Press, New York pp 265-288.

Girardier L, Seydoux J, Berger M, Veicsteinas A (1978) Selective pancreatic nerve section. J Physiol Paris 74: 731-735

Guicheney P, Meyer P (1979) Biochemical approach to pre- and postsynaptic α-adrenoceptors. Trends Pharmacol Sci 1: 69-70

Heding LG (1966) A simplified insulin radioimmunoassay method. In: Donato L, Milhaud G, Sirchis J (eds) Labelled proteins in tracer studies. Euratom Brussel, pp 345-350

Hertelendy F, Takahashi K, Machlin LJ, Kipnis DM (1970) The effect of chronic adrenergic blockade on the inhibiton by epinephrine of growth hormone and insulin release in sheep. Horm Metab Res 2: 257-259

Järhult J, Ahrén B, Lundquist I (1979) Inhibitory effect of somatostatin on insulin secretion during α-adrenergic blockade in three different species Acta Endocr (Kbh) 92: 166-173

Järhult J, Holst J (1979) The role of the adrenergic innervation to the pancreatic islets in the control of insulin release during exercise in man. Pflügers Arch 383: 41-45

Kostrzewa RM, Jacobowitz DM (1974) Pharmacological actions of 6-hydroxydopamine. Pharmacol Rev 26: 199-288

Langer SZ (1974) Presynaptic regulation of catecholamine release. Biochem Pharmacol 23: 1793-1800

Langer SZ (1977) Presynaptic receptors and their role in the regulation of transmitter release. Br J Pharmacol 60: 481-497

Larsson LI, Sundler F, Håkanson R (1976) Pancreatic polypeptide - a postulated new hormone: Identification of its cellular storage site by light and electron microscopic immunocytochemistry. Diabetologia 12: 211-226

Lorén I, Björklund A, Falck B, Lindvall O (1980a) The aluminium-formaldehyde (ALFA) histofluorescence method for improved visualization of catecholamines and indoleamines. I. A detailed account of the methodology for central nervous tissue using paraffin, cryostat or vibratome sections. J Neurosci Meth 2: 277-300

Lorén I, Schmidt RM, Björklund A, Lindvall O, Falck B (1980b) An improved method for monoamine histofluorescence in neo- and postnatal brain. To be published

Loubatières-Mariani MM, Chapal J, Ribes G, Loubatières AL (1977) Discrepancies in the response of the insulin secreting cells of the dog and rat to different adrenergic stimulating agents. Acta Diabetol Lat 14:144-155.

Lundquist I (1971) Insulin secretion. Its regulation by monoamines and acid amyloglucosidase. Acta Physiol Scand Suppl 372: 5-47

Lundquist I (1972a) Interaction of amines and aminergic blocking agents with blood glucose regulation. I. β-Adrenergic blockade. Europ J Pharmacol 18: 213-224

Lundquist I (1972b) Interaction of amines and aminergic blocking agents with blood glucose regulation. II. α-Adrenergic blockade. Europ J Pharmacol 18: 225-235

Lundquist I, Ericson LE (1978) β-Adrenergic insulin release and adrenergic innervation of mouse pancreatic islets. Cell Tiss Res 193: 73-85

Lundquist I, Sundler, F, Ahrén B, Alumets J, Håkanson R (1979) Somatostatin, pancreatic polypeptide, substance P, and neurotensin: Cellular distribution and effects on insulin secretion in the mouse. Endocrinology 104:832-838.

Porte Jr D (1967) A receptor mechanism for the inhibition of insulin release by epinephrine in man. J clin Invest 46: 86-94

Porte Jr D, Williams RH (1966) Inhibiton of insulin release by norepinephrine in man. Science 152: 1248-1250

Rerup C, Lundquist I (1966) Blood glucose level in mice. I. Evaluation of a new technique of multiple serial sampling. Acta Endocrinol (Kbs) 52: 357-367

Schalch DS (1967) The influence of physical stress and exercise on growth hormone and insulin secretion in man. J Lab Clin Med 69:256-269

Smith PH, Woods SC, Porte Jr D (1976) Phentolamine blocks the somatostatin--mediated inhibition of insulin secretion. Endocrinology 98: 1073-1076

Werrbach JH, Gale CC, Goodner CJ, Conway MJ (1970) Effects of autonomic blocking agents on growth hormone, insulin, free fatty acids and glucose in baboons. Endocrinology 86: 77-82

Woods SC, Porte Jr D (1974) Neural control of the endocrine pancreas. Physiol Rev 54: 596-619

Wright PH, Malaisse WJ (1968) Effects of epinephrine, stress and exercise on insulin secretion by the rat. Amer J Physiol 214: 1031-1034

Adrenalectomy and Chemical Sympathectomy by 6-Hydroxydopamine: Effects on Basal and Stimulated Insulin Secretion

Bo Ahrén and Ingmar Lundquist

Department of Pharmacology, University of Lund, Lund, Sweden

ABSTRACT

Influences of the sympatho-adrenal system on basal and stimulated insulin secretion were studied in vivo in the conscious mouse and rat. In the mouse, adrenalectomy or chemical sympathectomy, induced by 6-hydroxydopamine, lowered basal insulin concentrations moderately. A marked depression of basal insulin concentration (about 50%) was seen after the combined treatment of chemical sympathectomy and adrenalectomy. In short-term experiments in mice, insulin secretion stimulated by glucose or the cholinergic agonist carbachol was enhanced after chemical sympathectomy and/or adrenalectomy, whereas that to the synthetic octapeptide of cholecystokinin (CCK-8) was inhibited. The influences on the glucose effect took time to develop, whereas those on the effect of CCK-8 vanished with time. Terbutaline-induced insulin secretion was increased after chemical sympathectomy, but decreased after chemical sympathectomy plus adrenalectomy.

The glucose elimination rate after 6 weeks of chemical sympathectomy was increased in mice and decreased in rats. The insulin response to glucose was enhanced in mice, but it seemed diminished in rats after long-term sympathectomy.

In conclusion, the sympatho-adrenal system is involved in regulation of basal insulin concentrations in the mouse, and seems to be of importance for stimulated insulin secretion; the influence being dependent on the nature of the secretagogue.

INTRODUCTION

It is well established that adrenergic nerves innervate the pancreatic islets (6, 7, 15) and several studies have demonstrated a possible role of these nerves in the regulation of insulin secretion. Thus, electrical stimulation of the splanchnic nerves in the calf, dog, sheep and cat seems to inhibit insulin secretion (2, 16, 20), and reflex stimulation of the sympathetic system by exercise in man and baroreceptor unloading in the cat also depress the release of insulin (11, 12). Moreover, surgical splanch-

nic denervation and chemical sympathectomy in the rat elevate the basal insulin concentration (1, 5). In addition, noradrenaline and adrenaline have been shown to have the capability to inhibit insulin secretion through their α-adrenoceptor stimulatory property (17, 19) whereas β-adrenoceptor stimulation initiates insulin secretion (18). Thus, a vast amount of evidence shows that the sympatho-adrenal system, which consists of the sympathetic nerves and the adrenal medullary hormones, can influence basal insulin secretion (cf. 8).

The present study was undertaken in order to further study the influence of the sympatho-adrenal system on insulin secretion with special attention to its influence on stimulated insulin release.

MATERIALS AND METHODS

Animals. Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25-35 g, and female rats of the Wistar strain (Møllegaard Avlslaboratorium, Skensved, Denmark), weighing 250-300 g, were used throughout the experiments. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum before and during the experiments. Adrenalectomy was performed by the lumbar approach under light ether anaesthesia. Following adrenalectomy the mice were maintained on the usual diet and given 0.154 mol/l saline to drink.

Drugs. 6-Hydroxydopamine was from Hässle AB, Mölndal, Sweden, ascorbic acid from E. Merck AG, Darmstadt, BRD, terbutaline (2-tert-butylamino-(3,5-dihydroxyphenyl)ethanolsulfate) from Draco AB, Lund, Sweden, synthetic octapeptide of cholecystokinin (CCK-8), (Kinevac®) from Squibb, New Brunnswick, N.J., USA, D-glucose and carbachol from British Drug Houses Ltd, Poole, England.

Experiments. Fourteen days after the adrenalectomy, adrenalectomized and intact mice were treated with an i.v. injection of 0.154 mol/l saline or 6-hydroxydopamine (0.19 mmol/kg), which was dissolved in ice-cold 0.154 mol/l saline containing 56 mmol/l ascorbic acid. This dose of 6-hydroxydopamine has recently been shown to induce a substantial reduction of islet adrenergic nerves in the mouse (to be published). Two or 8 days later the mice were subjected to a rapid i.v. injection of equipotent doses of D-glucose (2.8 mmol/kg), terbutaline (3.6 μmol/kg), CCK-8 (6.3 nmol/kg) or carbachol (160 nmol/kg), or saline. Ten μl/g body weight was given. Two (D-glucose, CCK-8 and carbachol) or 5.5 (terbutaline) min later blood was sampled by orbital puncture (21). Previous experiments have shown that peak levels of plasma concentrations of immunoreactive insulin in normal mice occur at these times after a rapid i.v. injection, and the time intervals to the peak levels were not altered after manipulations with the sympatho-adrenal system.

In a long-term experiment, 6-hydroxydopamine was injected i.v. to intact mice (0.19 mmol/kg) and rats (0.32 mmol/kg). Six weeks later D-glucose (2.8 mmol/kg) was injected i.v. and blood was taken immediately before the injection and at different time intervals thereafter.

Determination of Insulin and Glucose. The concentrations of immunoreactive insulin (IRI) in plasma were determined by radioimmunoassay (10) and plasma glucose concentrations by a glucose oxidase method (4).

Statistics. The results are expressed as mean ± SEM. The increase in plasma IRI in response to the secretagogues was calculated by subtracting the plasma IRI concentration in the saline-treated control mice from those treated with a secretagogue and by calculating the weighted SEM, taking

into account SEM of both groups. The glucose elimination rate (K-value) was calculated from the regression line of the blood glucose levels obtained after subtracting the basal glucose values.

RESULTS

In short-term experiments, mice were subjected to different manipulations of the sympatho-adrenal system (adrenalectomy and/or chemical sympathectomy). Fig. 1 shows the changes in the basal concentrations of plasma IRI and glucose in the different groups of mice. Adrenalectomy was performed 14 days prior to the chemical sympathectomy and the blood was sampled after another 2 or 8 days. It is seen that adrenalectomy depressed basal plasma IRI and glucose, and that chemical sympathectomy induced reduction of basal insulin secretion visible after 8 days.

Plasma glucose concentrations were slightly lower in response to chemical sympathectomy after two days, but plasma glucose concentrations after 8 days were not different from controls. The combined treatment of adrenalectomy and chemical sympathectomy lowered basal plasma IRI by about 50% and plasma glucose by about 15% 8 days after the sympathectomy.

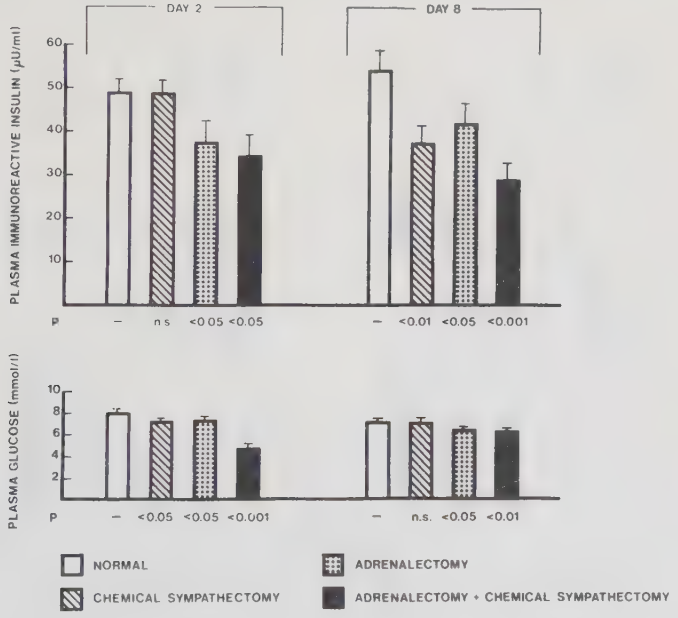


Fig. 1. Plasma concentrations of immunoreactive insulin (upper panel) and glucose (lower panel) in 4 groups of mice: a) normal mice injected with the vehicle for 6-hydroxydopamine, b) mice subjected to chemical sympathectomy by 6-hydroxydopamine 2 (left panel) or 8 (right panel) days prior to the blood sampling, c) mice adrenalectomized 14 days prior to the injection of the vehicle for 6-hydroxydopamine 2 (left panel) or 8 (right panel) days prior to the blood sampling, and d) mice adrenalectomized 14 days prior to the injection of 6-hydroxydopamine 2 (left panel) or 8 (right panel) days prior to the blood sampling. There were 19-27 animals in each group. Bars indicate SEMs, P probability level of random difference vs normal mice.

Fig. 2 illustrates the increase in plasma IRI concentrations after an i.v. injection of terbutaline, glucose, carbachol or CCK-8 in mice subjected to adrenalectomy and/or chemical sympathectomy and in control mice. The experiments were performed 2 days after 6-hydroxydopamine treatment. Terbutaline induced an insulin secretory response of about the same magnitude in all groups of mice, whereas the response to CCK-8 was decreased after adrenalectomy and/or chemical sympathectomy. The insulin secretory response to glucose was increased in mice subjected to the combined treatment of adrenalectomy and chemical sympathectomy, whereas either adrenalectomy or chemical sympathectomy alone did not influence the response to glucose. The cholinergic agonist carbachol induced a greater insulin secretion in adrenalectomized and/or chemically sympathectomized mice than in controls.

Plasma glucose concentrations were 8.0 $\pm$ 0.2 mmol/l in control mice (n =35), 7.4 $\pm$ 0.1 mmol/l in chemically sympathectomized mice (n = 27), 7.2 $\pm$ 0.1 mmol/l in adrenalectomized mice (n = 28), and 4.2 $\pm$ 0.2 mmol/l in adrenalectomized-sympathectomized mice (n = 23) after injection of saline. There were no difference from these values among mice treated with terbutaline, CCK-8 or carbachol. Injection of glucose increased plasma glucose to 15.0 $\pm$ 0.2 mmol/l in normal mice, 13.7 $\pm$ 0.3 mmol/l in chemically sympathectomized mice, 14.0 $\pm$ 0.3 mmol/l in adrenalectomized mice, and to 11.0 $\pm$ 0.4 mmol/l in adrenalectomized-sympathectomized mice.

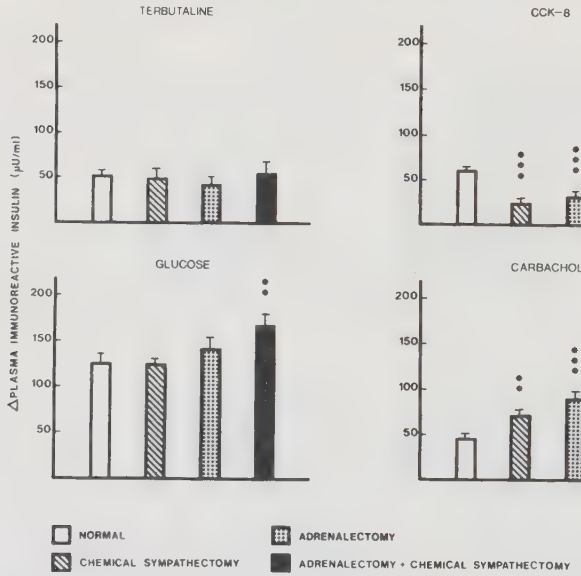


Fig. 2. Increase in plasma immunoreactive insulin concentrations (peak level) after the i.v. injection of terbutaline (3.6 μmol/kg), carbachol (0.16 μmol/kg), glucose (2.8 mmol/kg), or CCK-8 (6.3 nmol/kg) in mice injected with the vehicle for 6-hydroxydopamine (normal) or with 6-hydroxydopamine (chemical sympathectomy) 2 days prior to the experiments. Adrenalectomy was performed 16 days prior to the experiments. There were 12-23 animals in each group. Bars indicate SEMs, asterisks probability level of random difference vs normal mice (* $p < 0.01$, ** $p < 0.001$).

Fig. 3 shows the increase in plasma IRI after treatment with terbutaline, glucose, CCK-8 or carbachol in intact, adrenalectomized and/or chemically sympathectomized mice. In this experimental series, 6-hydroxydopamine was

given 8 days prior to the experiments. The insulin response to terbutaline was increased in mice subjected to chemical sympathectomy, and decreased in mice subjected to the combined treatment of adrenalectomy and chemical sympathectomy, whereas the response to CCK-8 was decreased in chemically sympathectomized mice and unchanged after adrenalectomy or combined treatment. Glucose-induced insulin secretion was potentiated by adrenalectomy alone and by the combined treatment of adrenalectomy and chemical sympathectomy and the insulin secretory response to carbachol was increased in all three groups of mice subjected to manipulations of the sympatho-adrenal system compared to intact mice. Plasma glucose concentrations were as follows after injection with saline: control mice 8.1 $\pm$ 0.2 mmol/l (n = 35), sympathectomized mice 8.5 $\pm$ 0.4 mmol/l (n = 33), adrenalectomized mice 7.7 $\pm$ 0.4 mmol/l (n = 33), adrenalectomized-sympathectomized mice 7.3 $\pm$ 0.5 mmol/l (n = 27). Terbutaline, CCK-8 and carbachol did not change these values, and glucose injection elevated them to 14.6 $\pm$ 0.5 mmol/l, 15.1 $\pm$ 0.5 mmol/l, 13.6 $\pm$ 0.4 mmol/l and 13.2 $\pm$ 0.4 mmol, respectively.

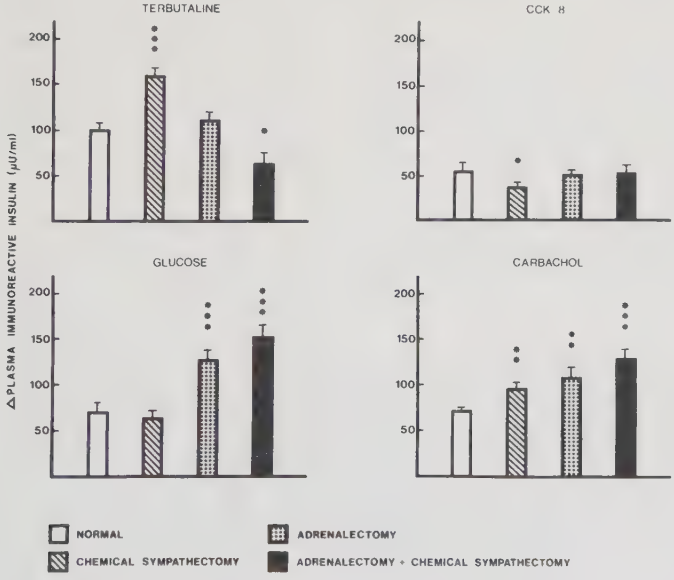


Fig. 3. Increase in plasma immunoreactive insulin concentrations (peak level) after the i.v. injection of terbutaline (3.6 μmol/kg), carbachol (0.16 μmol/kg), glucose (2.8 mmol/kg), or CCK-8 (6.3 nmol/kg) in mice injected with the vehicle for 6-hydroxydopamine (normal) or with 6-hydroxydopamine (chemical sympathectomy) 8 days prior to the experiments. Adrenalectomy was performed 22 days prior to the experiments. There were 10-23 animals in each group. Bars indicate SEMs, asterisks probability level of random difference vs normal mice (* $p < 0.01$, ** $p < 0.001$).

A long-term experiment was performed in mice and rats chemically sympathectomized 6 weeks earlier. These animals were subjected to an intravenous glucose tolerance test (fig. 4). It is seen that the glucose disappearance rate was increased in mice and decreased in rats subjected to chemical sympathectomy compared to normal animals. The glucose elimination rate (K-value) was 3.0 $\pm$ 0.4 in normal mice and 4.3 $\pm$ 0.3 in sympathectomized mice ($p < 0.05$). The values in the rat were 3.2 $\pm$ 0.2 in normal and 2.5 $\pm$ 0.1 in sympathectomized ($p < 0.05$). Basal plasma IRI concentrations were slightly lower in the chemically sympathectomized mice ($p < 0.05$) whereas no

significant difference was seen in rats. Plasma IRI increased significantly more ($p < 0.05$) in sympathectomized mice than in control mice during the first two minutes after the glucose injection, whereas there was no significant difference in the two rat groups regarding acute secretion of insulin. However, 15 minutes after the glucose injection, chemically sympathectomized rats had lower plasma IRI levels than controls ($p < 0.01$).

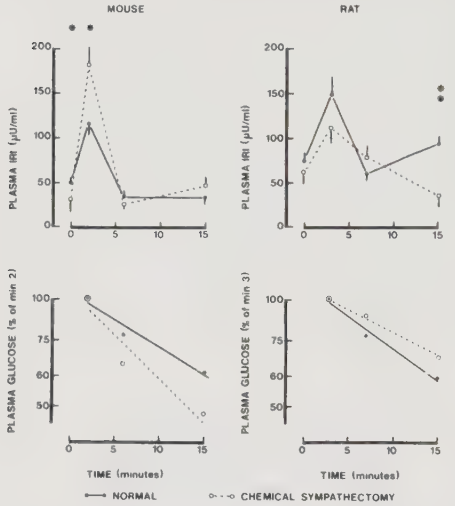


Fig. 4

Plasma concentrations of immunoreactive insulin (upper panel) and glucose elimination rate (lower panel, log scale) in normal and chemically sympathectomized mouse (left panel) and rat (right panel) after the i.v. injection of glucose, 2.8 mmol/kg. There were 6 animals in each group. Chemical sympathectomy was performed by 6-hydroxydopamine 6 weeks prior to the experiments. Bars indicate SEMs, asterisks probability level of random difference (* $p < 0.05$, ** $p < 0.01$).

DISCUSSION

Since 6-hydroxydopamine is selectively taken up by adrenergic nerves and destroys the nerve terminals (13) pretreatment with this drug leads to chemical sympathectomy. The doses of 6-hydroxydopamine used in the present study have recently been shown to induce a reduction of islet adrenergic nerves, leaving the insulin cells unaffected (to be published). This study shows that the sympatho-adrenal system is of importance for basal insulin secretion in the mouse since fourteen days after adrenalectomy and 8 days after chemical sympathectomy basal plasma insulin was slightly decreased. Plasma glucose was slightly depressed by adrenalectomy and initially by the chemical sympathectomy.

Previously the decrease in basal insulin after adrenalectomy has been reported (14). In earlier studies an increase in basal insulin after surgical or chemical sympathectomy was seen in the rat (1, 5) but no change after sympathectomy in man (3) or dog (9). Thus, species differences may exist concerning the role of the sympatho-adrenal system for regulation of basal insulin and a species difference between mouse and rat is shown in the present study. The flattened glucose disappearance rate in the glucose tolerance test in chemically sympathectomized rats confirms an earlier study (5) and is different from the increased glucose elimination rate in the mouse. Glucose-induced insulin secretion was enhanced in mice and decreased in rats, after long-term chemical sympathectomy.

Beside the regulating effect on insulin secretion under basal conditions the sympatho-adrenal system seems to modulate the insulin secretory response to different secretagogues. It is conceivable that the four secretagogues used in the present study, the β_2-adrenoceptor stimulator terbuta-

line, the cholinergic agonist carbachol, the C-terminal octapeptide of cholecystokinin (CCK-8) and glucose, stimulate insulin secretion through different mechanisms of action, and some of these mechanisms thus seem to be coupled to sympatho-adrenergic activity.

The cholinergic agonist carbachol induced a more pronounced insulin secretion after chemical sympathectomy and adrenalectomy. It has been demonstrated that cholinergic agents can liberate catecholamines from nerve terminals (22) and perhaps may these amines by their α-adrenergic effect counteract the direct stimulatory effect by carbachol on insulin secretion in intact animals, thus diminishing the true response. After abolishing this counteraction the observed insulin release thus may become greater. Such an explanation is, however, less probable in sympathectomized mice with intact adrenals, since the insulin secretory response to carbachol is very rapid.

CCK-8 had a weaker insulin-stimulatory effect in mice subjected to adrenalectomy and/or sympathectomy than in controls but the inhibition of CCK--induced insulin secretion by elimination of the sympatho-adrenal system vanished with time. On the other hand, glucose-induced insulin secretion seemed to be increased after elimination of the sympatho-adrenal system. This increase developed slowly. In the earliest experiments the increase was visible only after the combined treatment of adrenalectomy and chemical sympathectomy; after another week it could be seen also after adrenalectomy, and in the long-term experiment, glucose-induced insulin secretion was increased also after chemical sympathectomy.

The insulin secretory response to β_2-adrenoceptor stimulation by terbutaline was increased by 6-hydroxydopamine, which might be due to a local supersensitivity after sympathectomy. Contrary, adrenalectomy plus chemical sympathectomy was followed by a decreased response to terbutaline. This might reflect a certain dependency of a critical amount of catecholamines for the insulin response to this drug.

The exact mechanisms behind the changes in the insulin secretory response to these different secretagogues remain to be elucidated. Beside the influences of the adrenergic system also changes exerted by altered plasma glucose and direct effects of 6-hydroxydopamine have to be considered. However, a general inhibitory influence on the insulin cell is apparently not exerted by the adrenergic system, rather it seems as if specialized functions are maintained by it, and when eliminating these influences changes in the insulin cell responsiveness occur, which lead to differential effects on stimulated insulin secretion depending on the nature of the secretagogues. It is also obvious from these studies that the influences on stimulated insulin secretion was dependent on the time lapse after manipulations with the sympatho-adrenal system.

ACKNOWLEDGEMENTS

The authors express their appreciation to Lena Kvist and Peter Okmark for technical assistance and to Eva Björkbom for secretarial help.

This study was supported by the Swedish Medical Research Council (04P--4289, 14X-4289) and by grants from the Medical Faculty, University of Lund, Lund, Sweden.

REFERENCES

1. Ahrén, B., Järhult, J., Lundquist, I.: Influence of the sympatho-adrenal system and somatostatin on the secretion of insulin in the rat. J. Physiol (Lond.) in press (1981).
2. Bloom, S.R., Edwards, A.V.: The release of pancreatic glucagon and inhibition of insulin in response to stimulation of the sympathetic innervation. J. Physiol. (Lond.) 253, 157-173 (1975).
3. Brodows, R.G., Pi-Sunyer, F.X., Campbell, R.G.: Insulin secretion in adrenergic insufficiency in man. J. Clin. Endocrinol. Metab. 38, 1103-1108 (1974).
4. Bruss, M.L., Black, A.L.: Enzymatic microdetermination of glycogen. Anal. Biochem. 84, 309-312 (1978).
5. Burr, I.M., Jackson, A., Culbert, S., Sharp, R., Felts, P., Olson, W.: Glucose intolerance and impaired insulin release following 6-hydroxydopamine administration to intact rats. Endocrinology 94, 1072-1076 (1974).
6. Cegrell, L.: The occurence of biogenic monoamines in the mammalian endocrine pancreas. Acta Physiol. Scand. Suppl. 314, 1-60 (1968).
7. Esterhuizen, A.C., Spriggs, T.L.B., Lever, M.D.: Nature of islet-cell innervation in the cat pancreas. Diabetes 17, 33-36 (1968).
8. Gerich, J.E., Lorenzi, M.: The role of the autonomic nervous system and somatostatin in the control of insulin and glucagon secretion. In: Ganong, W.F., Martini, L: Front. Neuroendocrinol, Vol. 5. Raven Press, New York, 1978, pp 265-288.
9. Girardier, L., Seydoux, J., Berger, M., Veicsteinas, A.: Selective pancreatic nerve section. An investigation of neural control of glucagon release in the conscious unrestrained dog. J. Physiol. (Paris) 74, 731-735 (1978).
10. Heding, L.: A simplified insulin radioimmunoassay method. In: Donato, L., Milhaud, G., Sirchis, J.: Labelled proteins in tracer studies. Euratom, Brussel, 1966, pp 345-350.
11. Järhult, J., Holst, J.J.: Reflex adrenergic control of endocrine pancreas evoked by unloading of carotid baroreceptors in cats. Acta Physiol. Scand. 104, 188-202 (1978).
12. Järhult, J., Holst, J.: The role of the adrenergic innervation to the pancreatic islets in the control of insulin release during exercise in man. Pflügers Arch. 383, 41-45 (1979).
13. Kostrzewa, R.M., Jacobowitz, D.M.: Pharmacological actions of 6-hydroxydopamine. Pharmacol. Rev. 26, 199-288 (1974).
14. Lundquist, I.: Interaction of amines and aminergic blocking agents with blood glucose regulation. II. α-Adrenergic blockade. Europ. J. Pharmacol. 18. 225-235 (1972).
15. Lundquist, I., Ericson, L.E.: β-Adrenergic insulin release and adrenergic innervation of mouse pancretic islets. Cell Tiss. Res. 193, 73-85 (1978).
16. Miller, R.E.: Neural inhibition of insulin secretion from the isolated canine pancreas. Am. J. Physiol. 229, 144-149 (1975).
17. Porte Jr, D., Williams, R.H.: Inhibition of insulin release by norepinephrine in man. Science 152, 1248-1250 (1966).
18. Porte Jr, D.: Beta adrenergic stimulation of insulin release in man. Diabetes 16, 150-155 (1967).
19. Porte Jr, D.: A receptor mechanism for the inhibition of insulin release by epinephrine in man. J. Clin. Invest. 46, 86-94 (1967).
20. Porte Jr, D., Girardier, L., Seydoux, J., Kanazawa, Y., Posternak, J.: Neural regulation of insulin secretion in the dog. J. Clin. Invest. 52, 210-214 (1973).

21. Rerup, C., Lundquist, I.: Blood glucose level in mice. I. Evaluation of a new technique of multiple serial sampling. Acta Endocrinol. (Kbh) 52, 357-367 (1966).
22. Westfall, T.C.: Local regulation of adrenergic neurotransmission. Physiol. Rev. 57, 659-728 (1977).

Effects of Selective and Nonselective β-Adrenergic Agents on Insulin Secretion in Vivo

Bo Ahrén and Ingmar Lundquist

Department of Pharmacology, University of Lund, Lund, Sweden

SUMMARY

Effects of various β-adrenergic agents on insulin secretion were investigated in vivo in mice. The nonselective β-stimulator isopropylnoradrenaline and the selective β_2-stimulator terbutaline both markedly and with the same efficacy stimulated insulin secretion in a dose-dependent manner; peak levels of plasma insulin after these two β-agonists were achieved at a later time point (5-6 min) than after stimulation with glucose or carbachol (1.5-2.5 min). At very high dose levels the β_1-stimulator isopropylaminothiazoloxypropanol slightly increased plasma insulin concentrations. The nonselective β-blocker propranolol and the β_2-selective blocker ICI 118,551 inhibited terbutaline-induced insulin release markedly and at comparable low dose levels, whereas the selective β_1-blocker metoprolol exerted this effect only at a high dose level. At higher dose levels these three blockers moderately depressed the insulin response to glucose suggesting a partial dependence on intact β-adrenoceptors for the effect of glucose. The β_2-blocker butoxamine and the β_1-blocker pamatolol did not influence insulin secretion. In conclusion, β-adrenoceptor stimulation enhances insulin secretion in vivo, but the β-adrenoceptors regulating insulin secretion do not fit excellently into conventional subdivision in β_1 and β_2, though they apparently are mainly of the β_2-type.

INTRODUCTION

Adrenaline and noradrenaline are known to stimulate α-adrenergic receptors and thereby inhibit insulin secretion (Coore and Randle, 1964; Porte, et al., 1966; Porte, 1969; Smith and Porte, 1976), and after blockade of the α-adrenoceptors by phentolamine these catecholamines induce stimulation of insulin secretion (Porte, 1967a; Lundquist, 1972b). Thus it has been proposed that insulin cells are equipped with stimulatory β- as well as inhibitory α-adrenergic receptors.

Several synthetic β-adrenergic agonists, such as isopropylnoradrenaline, salbutamol, or ritodrine, stimulate insulin release (Porte, 1967b; Loubatière, 1971; Lundquist and Ericson, 1978; Lenz et al., 1979), and it

has been suggested that the ß-adrenoceptors on the insulin cells are of the $ß_2$-type (Loubatières, 1971).

The present study was undertaken in order to study in more detail the relation of the ß-adrenergic receptors to insulin release, with special attention to the suggested subdivision of the ß-adrenergic receptors (Lands et al., 1967). Thus, the effects of nonselective ß-adrenergic stimulation as well as of selective $ß_1$- and $ß_2$-agonists on insulin release were studied. Furthermore, the influence of nonselective and selective $ß_1$- and $ß_2$-antagonists on basal and stimulated insulin secretion was investigated.

MATERIALS AND METHODS

Animals

Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25-35 g were used throughout the experiments. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden), and tap water ad libitum before and during the experiments.

Drugs

DL-terbutaline sulfate (1-(3,5-dihydroxyphenyl)-2-(tert-butylamino)ethanol sulfate) was a gift from Draco AB, Lund, Sweden, DL-isopropylnoradrenaline sulfate (1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol sulfate), DL-pamatolol sulfate (methyl- p- 2-hydroxy-3-(isopropylamino)proxy phenylethyl carbamate sulfate), DL-metoprolol tartrate (1-isopropylamino-3-(4-(2-methoxyethyl)-phenoxy)-2-propanol tartrate) and DL-ITP hydrochloride (DL-1-isopropylamino-3-(2-thiazoloxy)-2-propanol hydrochloride) were given by Hässle AB, Mölndal, Sweden, DL-propranolol hydrochloride (1-(isopropylamino)-3-(1-naphtyloxy)-2-propanol hydrochloride) and the drug ICI 118,551 DL-(erythro-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol) were supplied by ICI Ltd, Macclesfield, England, DL-butoxamine hydrochloride (erythro--1-(2,5-dimethoxyphenyl)-2-(tert-butylamino)propanol hydrochloride) was from Burroughs Wellcome & Co, London, England, and D-glucose and carbachol from British Drug Houses Ltd, Poole, England. All drugs were dissolved in 0.9% NaCl.

Experiments

Effects of ß-Adrenergic Agonists, Glucose and Carbachol on Insulin Secretion. Each of the three different ß-adrenergic agonists, terbutaline, isopropylnoradrenaline and ITP, was administered intravenously in a tail vein; 10 µl/g body weight was injected rapidly. Several different doses were given. In another series of experiments half-maximal doses of glucose (2.78 mmol/kg) or the cholinergic agonist carbachol (0.16 µmol/kg) was injected intravenously. Immediately before, and 2, 5.5 and 15 minutes after the injection, blood was drawn by orbital puncture using commercial constriction pipettes (Rerup and Lundquist, 1966). The mice were unanaesthetized all the time.

Effects of ß-Adrenergic Antagonists on Basal and Terbutaline-Induced Insulin Secretion. Each of the ß-adrenergic antagonists propranolol, butoxamine, ICI 118,551, metoprolol, and pamatolol, or saline, was injected intraperitoneally; different doses were given. Twenty minutes later terbutaline, 3.6 µmol/kg body weight, or saline, was given intravenously; 10 µl/g body weight was given i.p. as well as i.v.; 5.5 minutes after the i.v. injection

(peak levels of plasma insulin) blood was collected by orbital puncture.

Effects of β-Adrenergic Antagonists on Glucose-Induced Insulin Secretion. Each of the β-adrenergic antagonists propranolol, butoxamine, ICI 118,551, metoprolol, pamatolol or saline was injected intraperitoneally; different doses were given. Twenty minutes later glucose, 2.78 mmol/kg, was given intravenously; 10 µl/g was given i.p. as well as i.v.; 2 minutes after the i.v. injection (peak levels of plasma insulin) blood was collected by orbital puncture.

Determination of Insulin and Glucose

The concentration of immunoreactive insulin (IRI) in plasma was determined by radioimmunoassay (Heding, 1966), using ^{125}I-labelled pig insulin and guinea pig anti-pig insulin. The plasma glucose concentration was determined enzymatically (Bruss and Black, 1978).

Statistics

Means and SEMs are given. Student's t-test was employed for tests of significance.

RESULTS

Effects of β-Adrenergic Agonists, Glucose or Carbachol on Insulin Secretion
The three different β-adrenoceptor agonists, isopropylnoradrenaline, terbutaline, and ITP, respectively, was injected i.v. and blood samples were taken at 5.5 min. Fig. 1 shows that the non-selective β-agonist isopropylnoradrenaline and the selective β_2-agonist terbutaline both markedly stimulated insulin secretion in a dose-dependent manner. The maximal responses achieved by the two agonists were of the same magnitude; isopropylnoradrenaline being about 5-6 times more potent at equimolar, submaximal doses. The half-maximal response to terbutaline, about 100 µU/ml, was obtained after injection of 3.6 µmol/kg, and this dose was selected for the experiments with the different β-blockers. The selective β_1-agonist ITP slightly increased plasma immunoreactive insulin concentrations, but this effect was seen only at very high doses (Fig. 1).

Plasma glucose concentrations were 8.5 $\pm$ 0.3 mmol/l in control mice. There was no difference in plasma glucose between control mice and mice treated with the various β-adrenoceptor agonists.

Fig. 2 shows plasma immunoreactive insulin and glucose concentrations during 15 minutes following injection of half-maximal doses of terbutaline, glucose, carbachol, and saline, respectively. It is seen that the peak level in plasma insulin was obtained about 5-6 minutes after injection of terbutaline, and about 2 minutes after injection of glucose and carbachol, respectively. The time course pattern for isopropylnoradrenaline-stimulated insulin secretion was similar to that of terbutaline-induced (data not shown). Plasma glucose concentration was slightly depressed after 15 minutes in the terbutaline group, and this could probably be ascribed to the increased insulin release.

Effects of β-Adrenergic Antagonists on Basal and Stimulated Insulin Secretion

The β-antagonists were injected i.p. 20 minutes prior to an injection of terbutaline, glucose, or saline. Fig. 3 shows the effects of the nonselective β-blocker propranolol, 18 and 1.8 µmol/kg, on basal and stimulated insulin release. It is seen that propranolol at the studied dose-level

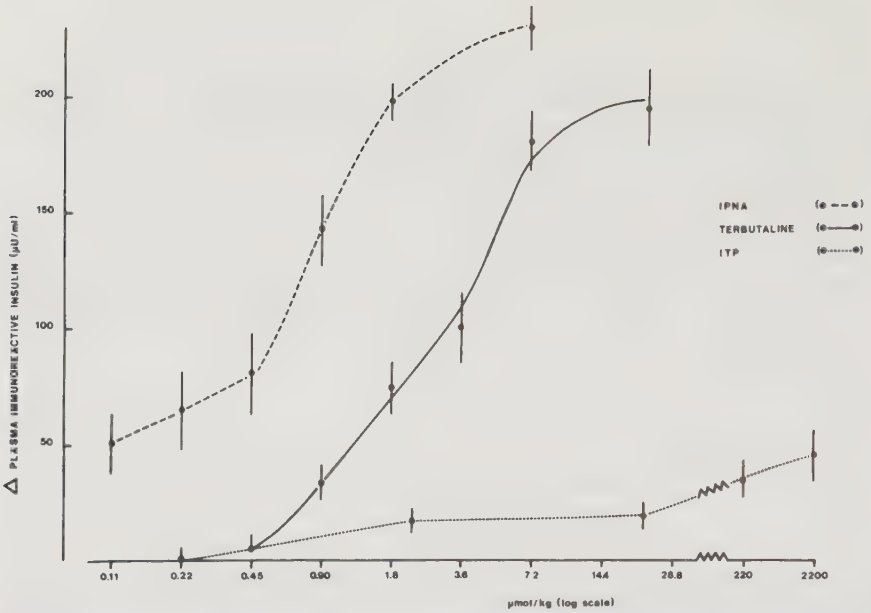


Fig. 1. Dose-response relationships between dose of terbutaline, isopropylnoradrenaline (IPNA), or isopropylthiazoloxypropanol (ITP), respectively, and insulin release. Plasma concentrations of immunoreactive insulin were measured in samples taken 5.5 minutes after an i.v. injection of the 3 drugs, respectively. Means and SEMs are shown. There were 8-20 animals in each group. Abscissa: dose of drug (μmol/kg) (log scale). Ordinate: plasma concentrations of immunoreactive insulin (μU/ml).

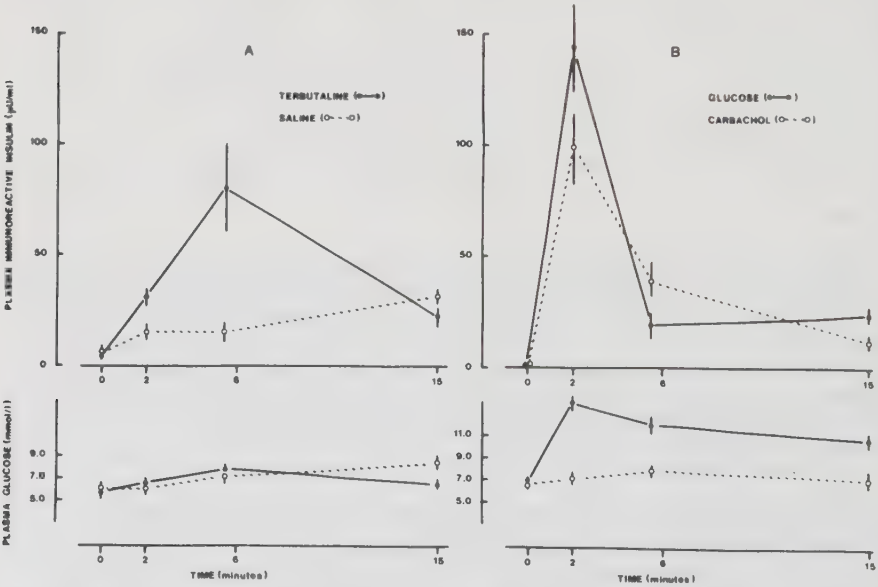


Fig. 2. Changes in plasma concentrations of immunoreactive insulin and glucose following an i.v. injection of terbutaline, 3.6 μmol/kg, and saline (Fig. 2A) and glucose, 2.78 mmol/kg, and carbachol, 0.16 μmol/kg (Fig. 2B), respectively. Each group of animals consisted of 10 mice. Mean and SEMs are shown.

had no effect on basal insulin concentrations, whereas it totally inhibited the insulin secretory response to terbutaline at both dose levels studied ($p < 0.001$). Terbutaline elevated plasma insulin concentrations to 143.8 ± 15.6 µU/ml ($p < 0.001$), and propranolol lowered this increase to 58.6 ± 7.9 µU/ml ($p < 0.001$) at the dose of 18 µmol/kg, and to 35.4 ± 8.9 µU/ml ($p < 0.001$) at the dose of 1.8 µmol/kg. Glucose enhanced plasma insulin concentrations to 142.1 ± 9.6 µU/ml ($p < 0.001$). Propranolol, 18 µmol/kg, depressed this increase to 82.7 ± 7.1 µU/ml ($p < 0.001$), whereas the lower dose of propranolol, 1.8 µmol/kg, was without effect on glucose-induced insulin secretion. Plasma glucose concentrations were 7.3 ± 0.2 mmol/l 5.5 min after terbutaline injection and 16.6 ± 0.3 mmol/l 2 min after glucose injection in the control groups. Propranolol did not induce any changes in plasma glucose concentration during the time-period studied, neither in the basal state, nor after injection of terbutaline or glucose.

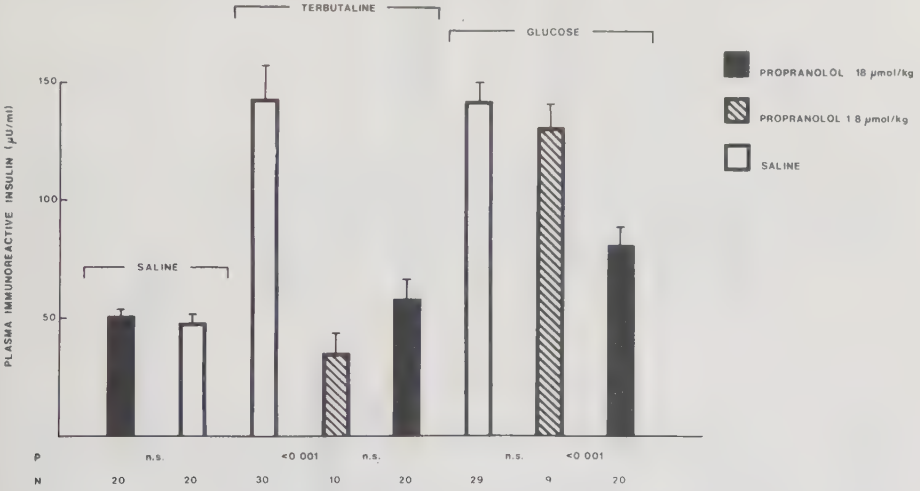


Fig. 3. Peak level of plasma concentrations of immunoreactive insulin after the i.v. injection of a half-maximal dose of terbutaline (3.6 µmol/kg) or glucose (2.78 mmol/kg). Pretreatment with saline or propranolol (18 or 1.8 µmol/kg) was given i.p. 20 minutes before the i.v. injection. Controls were given saline. Means and SEMs are shown. P indicates probability level of random difference. N.S. not significant. N number of animals.

Fig. 4 shows the effects of the selective β_2-antagonist butoxamine, 36 µmol/kg, on basal and stimulated insulin secretion. It is seen that β_2-blockade by butoxamine did not induce any changes in neither basal nor terbutaline-induced or glucose-induced secretion of insulin. There was no effect on plasma glucose concentrations by butoxamine in any group.

Fig. 5 shows the effects of the selective β_2-blocker ICI 118,551 on basal and stimulated insulin secretion. The drug had no effect on basal insulin secretion at the dose levels of 9 or 18 µmol/kg. Terbutaline enhanced plasma insulin to 145.1 ± 10.4 µU/ml ($p < 0.001$), and ICI 118,551 at the dose level of 9 µmol/kg depressed this increase to 71.2 ± 4.5 ($p < 0.001$) and at 1.5 µmol/kg to 63.8 ± 10.3 µU/ml ($p < 0.001$); i.e. terbutaline-induced insulin secretion was almost abolished. Glucose elevated plasma insulin levels to 143.5 ± 8.9 µU/ml ($p < 0.001$). ICI 118,551 at the dose level of 9 µmol/kg inhibited this increase to 87.2 ± 11.5 µU/ml ($p < 0.001$), whereas it had no effect on glucose-induced insulin secretion at the dose level of 1.5 µmol/kg. ICI 118,551 had no effect on plasma glucose concentrations in any of the groups.

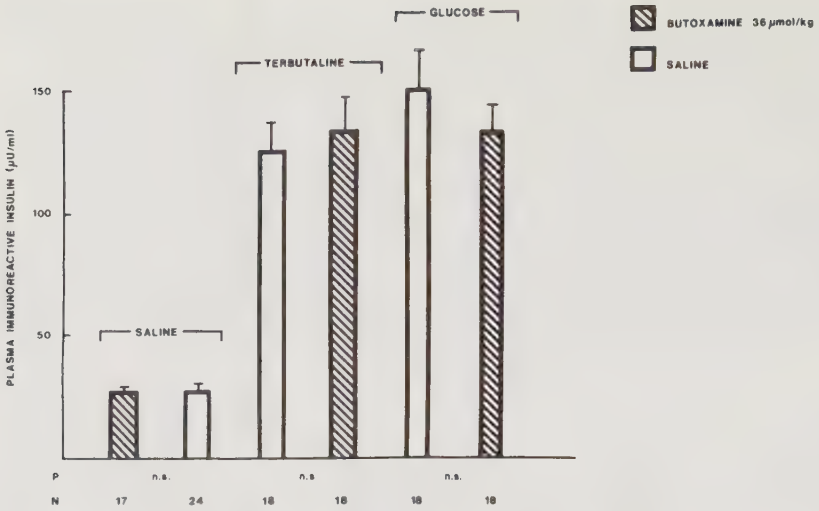


Fig. 4. Peak level of plasma concentrations of immunoreactive insulin after the i.v. injection of a half-maximal dose of terbutaline (3.6 µmol/kg) or glucose (2.78 mmol/kg). Pretreatment with saline or butoxamine (36 µmol/kg) was given i.p. 20 minutes before the i.v. injection. Controls were given saline. Means and SEMs are shown. P indicates probability level of random difference. N.S. not significant. N number of animals.

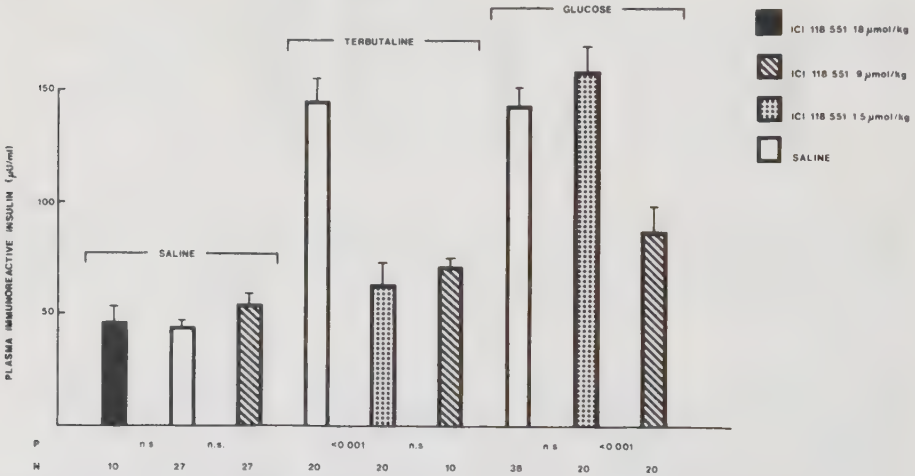


Fig. 5. Peak levels of plasma immunoreactive insulin after the i.v. injection of a half-maximal dose of terbutaline (3.6 µmol/kg) or glucose (2.78 mmol/kg). Pretreatment with saline or ICI 118,551 (18, 9 or 1.5 µmol/kg) was given i.p. 20 minutes before the i.v. injection. Controls were given saline. Means and SEMs are shown. P indicates probability level of random difference. N.S. not significant. N number of animals.

Fig. 6 shows the effects of the selective β_1-antagonist metoprolol on basal, terbutaline- and glucose-induced insulin secretion. It is seen in the figure that metoprolol depressed basal insulin secretion and inhibited the effect of terbutaline. At the higher dose level, 360 µmol/kg, the plasma insulin concentration after terbutaline, 132.3 $\pm$ 15.6 µU/ml, was depressed to 29.0 $\pm$ 9.1 µU/ml, i.e. by about 90 % ($p < 0.001$). A lower dose level of metoprolol, 36 µmol/kg, was found to inhibit terbutaline-

-induced insulin secretion by about 60% ($p < 0,001$); plasma insulin was 64.9 ± 5.8 µU/ml. The lowest studied dose level of metoprolol, 9 µmol/kg, had no effect on the insulin secretory response to terbutaline. Basal insulin secretion was depressed (by about 50%, $p < 0.001$) at the high dose level of metoprolol (360 µmol/kg) but uninfluenced at the other dose levels. The highest dose of metoprolol was also found to slightly depress the insulin response to glucose (by about 30%, $p < 0.001$) whereas the lower dose, 36 µmol/kg, was without effect. There was no difference in plasma glucose concentrations in animals pretreated with metoprolol compared with controls, neither in the basal state, nor after injection of terbutaline or glucose.

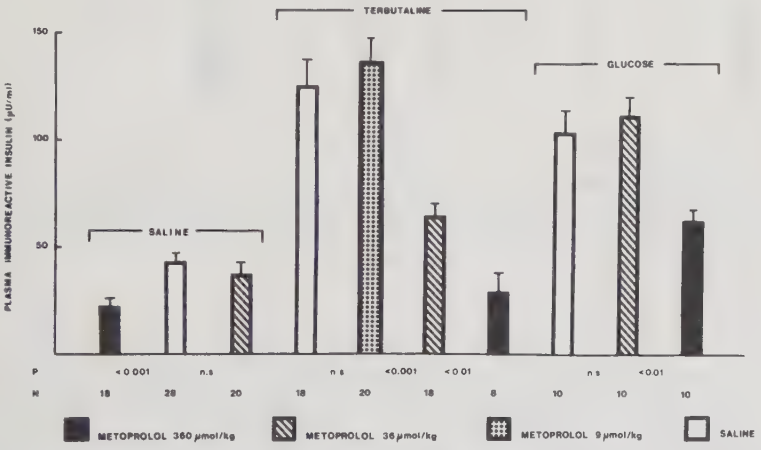


Fig. 6. Peak levels of plasma concentrations of immunoreactive insulin after the i.v. injection of a half-maximal dose of terbutaline (3.6 µmol/kg) or glucose (2.78 mmol/kg). Pretreatment with saline or metoprolol (360, 36, or 9 µmol/kg) was given i.p. 20 minutes before the i.v. injection. Controls were given saline. Means and SEMs are shown. P indicates probability level of random difference. N.S. not significant. N number of animals.

The effects of the selective β_1-blocker pamatolol on basal and stimulated insulin secretion are shown in Fig. 7. It is seen that pamatolol, 36 or 360 µmol/kg, did not influence basal insulin secretion, nor did pamatolol change the insulin secretory response to terbutaline or glucose. However, pamatolol influenced plasma glucose concentrations. The control group had a plasma glucose concentration of 7.4 ± 0.2 mmol/l and the group treated with the lower dose of pamatolol, 36 µmol/kg, a plasma glucose of 6.8 ± 0.2 mmol/l, and with the higher dose of pamatolol, 360 µmol/kg, a plasma glucose of 5.8 ± 0.1 mmol/l ($p < 0.001$). Furthermore, pamatolol, 360 µmol/kg, depressed plasma glucose concentration in terbutaline-treated animals. Control mice injected with terbutaline had a plasma glucose of 7.7 ± 0.1 mmol/kg compared to 6.8 ± 0.2 mmol/kg in animals treated with the high dose of pamatolol plus terbutaline ($p < 0.001$). The low dose of pamatolol did not change the plasma glucose after terbutaline. Thus, pamatolol lowered plasma glucose, and despite this exerted no influence on plasma insulin, neither in the basal state, nor after stimulation by terbutaline. Pamatolol did not influence the rise in plasma glucose concentrations seen after glucose injection.

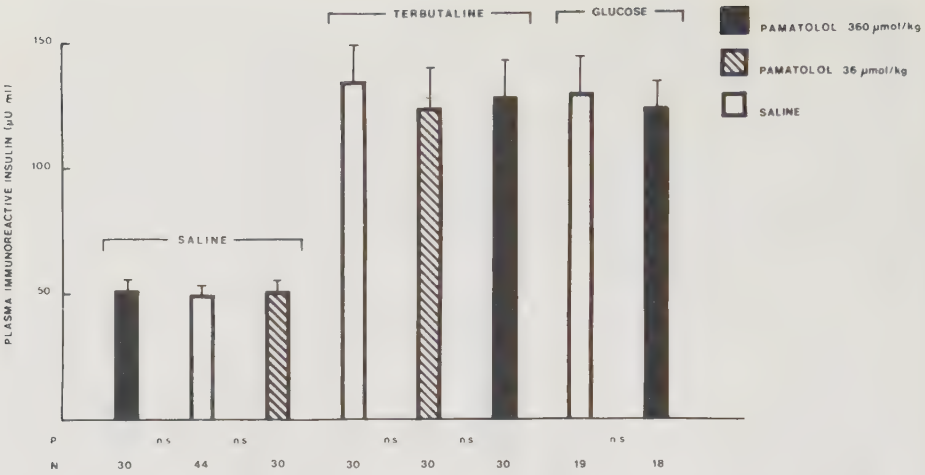


Fig. 7. Peak levels of plasma concentrations of immunoreactive insulin after the i.v. injection of a half-maximal dose of terbutaline (3.6 µmol/kg) or glucose (2.78 mmol/kg). Pretreatment with saline or pamatolol (360 or 36 µmol/kg) was given i.p. 20 minutes before the i.v. injection. Controls were given saline. Means and SEMs are shown. P indicates probability level of random difference. N.S. not significant. N number of animals.

DISCUSSION

Adrenergic agents are known to influence insulin secretion, and α-adrenergic agonists inhibit basal and glucose-induced insulin release *in vivo* as well as *in vitro* (Coore and Randle, 1964; Porte et al., 1966; Porte, 1967a; Malaisse et al., 1967). Contrary, β-adrenergic agonists stimulate insulin secretion. Thus, adrenaline stimulates insulin secretion during α-adrenergic blockade (Porte, 1967a; Malaisse et al., 1967; Lundquist, 1972b), and isopropylnoradrenaline induces release of insulin (Porte, 1967a; Loubatières et al., 1971; Furman and Tayo, 1974; Kaneto, Miki and Kosaka, 1975; Herrmann and Deckert, 1977; Loubatières-Mariani et al., 1977; Lundquist and Ericson, 1978; Irie et al., 1979).

The β-adrenoceptors have been subdivided into β_1- and β_2-subtypes, of which the β_1-type mediates adrenergic effects on the heart, intestine and lipolysis, and the β_2-type the effects on smooth muscle, glycogenolysis and glycolysis (Lands et al., 1967). It has been suggested from the results of several studies that the β-receptors regulating insulin secretion are of the β_2-type, since the selective β_1-blockers practolol and sotalol did not affect isopropylnoradrenaline-induced insulin release and the β_2-stimulators salbutamol, trimetoquinol, procaterol and ritodrine were found to be potent insulin stimulatory agents in dogs and man (Loubatières et al., 1971; Kaneto, Miki and Kosaka, 1975; Irie et al., 1979; Lenz et al., 1979). However, a previous study in the rat reported that selective β_1--blockers such as practolol and sotalol indeed had the ability to inhibit isopropylnoradrenaline-induced insulin secretion (Furman and Tayo, 1974).

In the present study we used three different β-adrenergic stimulators and five β-adrenergic blockers. Isopropylnoradrenaline stimulates both β_1- and β_2-adrenoceptors (Jenkinson, 1973), whereas terbutaline has been considered to be a selective β_2-agonist (Bergman, Persson and Wetterlin, 1969). Isopropylaminothiazoloxypropanol (ITP) stimulates selectively the heart muscle, and has therefore been classified as a selective β_1-agonist (Roszkowski et al., 1972). Among the blockers used in this study proprano-

lol is a nonselective ß-blocker (Jenkinson, 1973). Butoxamine and ICI 118,551 mainly inhibit the $ß_2$-adrenoceptors (Burns and Lemburger, 1965; Omini et al. 1979), whereas metoprolol and pamatolol (Åblad et al., 1975; Carruthers et al., 1978) are both $ß_1$-selective antagonists. However, it should be emphasized that the selectivity is more or less complete (Lefkowitz, 1975) and that the drugs may have actions unrelated to ß-adrenolytic activity. Thus, propranolol and ICI 118,551 have been found to have membrane stabilizing properties at comparatively low dose levels, whereas metoprolol and butoxamine exert local anaesthetic effects at high doses and pamatolol is practically devoid of such an effect (Howe and Shanks, 1966; Wilkenfeld and Levy, 1968; Åblad et al, 1975; Bilski et al., 1979; Dr. B. Lundgren, Hässle AB, Mölndal, Sweden, personal communication). In addition, blockers of the adrenergic receptors may also have intrinsic activity, and induce a stimulation of the receptor. Neither propranolol, ICI 118,551, metoprolol or pamatolol has, however, been found to have such intrinsic activity (Black, Duncan and Shanks, 1965; Johansson, 1979; Bilski et al., 1979).

Our results show that isopropylnoradrenaline and terbutaline both markedly stimulated insulin release, and that the efficacies of the two ß-agonists were of similar magnitude. However, in equimolar submaximal doses isopropylnoradrenaline was more potent. Contrary, the selective $ß_1$-agonist ITP had a very weak insulin releasing effect. These findings suggest that the ß-receptors involved in regulating the insulin cell are mainly of the $ß_2$-type.

The nonselective ß-blocker propranolol, the $ß_1$-blocker metoprolol and the $ß_2$-blocker ICI 118,551 depressed at different dose levels terbutaline- and glucose-induced insulin release, whereas the $ß_2$-blocker butoxamine or the $ß_1$-blocker pamatolol had no effect on stimulated insulin secretion. In addition, metoprolol depressed basal insulin concentrations at a high dose level. Neither of these ß-antagonists induced any appreciable changes in plasma glucose concentrations during the time-period studied except pamatolol, which in the high dose slightly depressed plasma glucose. It should be emphasized that similar effects of the drugs were seen at largely different dose levels, and it is apparent that propranolol and the selective $ß_2$-blocker ICI 118,551 exerted their inhibitory effects at lower dose levels than metoprolol. Furthermore, the inhibition of the insulin response to terbutaline was observed at markedly lower doses of the various ß--blockers than the insulin response to glucose. Also, terbutaline-induced insulin release was more sensitive to ß-blockade than basal insulin secretion. Thus it is obvious that the effect of terbutaline on the insulin cells is highly dependent on intact ß-adrenoceptors. Also glucose-induced insulin secretion seems to be dependent on intact ß-adrenoceptors, but to a far lesser degree than the response to terbutaline. Further, it cannot be excluded that a negative influence of the ß-blockers on glucose-induced insulin release is partially a consequence of a local anaesthetic property of the drugs.

The demonstrated inhibition of ß-adrenergically induced insulin release by propranolol is a confirmation of other studies (Porte, 1967b; Loubatières et al., 1971; Lundquist and Ericson, 1978). The levorotatory form of propranolol has earlier been found to depress basal insulin levels (Lundquist 1972a; Lundquist and Ericson, 1978), but the racemic form was found to be without such an effect (cf. Fig. 3) at comparable dose levels. Furthermore, the ß-blocker propranolol has earlier been shown to partially inhibit glucose-induced insulin release (Cerasi, Efendić and Luft, 1969) and it is here reported that also metoprolol and ICI 118,551 have the capability to exert this effect; higher doses than used for inhibition of

the effect of terbutaline were necessary, however, to detect this effect. We were unable to detect any insulin lowering effect of the β_1-blocker pamatolol, which has been shown to be more β_1-selective than metoprolol and atenolol (Johansson, 1979), or the β_2-blocker butoxamine. Thus, our studies indicate that the β-receptors involved in the insulin secretory response to terbutaline are not entirely of a pure β_2-subtype as defined from the studies which have characterized the various drugs, rather it seems as a population of receptors is involved which cannot be classified according to the conventional subdivision in β_1-β_2-types. In addition, the β-adrenoceptors regulating insulin secretion *in vivo* may be regarded as organ-specific as e.g. the classical β_2-adrenoceptor antagonist butoxamine has no influence on the effect of terbutaline. Further subdivision of β-receptors have been discussed, but more convincingly evidence is necessary for establishing such a subdivision (Furchgott, 1972).

The time course pattern of the insulin secretory response to β-adrenergic stimulation was found to be different from that to glucose or carbachol (cf. Fig. 2). This suggests that at least partially separate mechanisms behind the insulin releasing effect of β-adrenergic agents and the other secretagogues might exist. Other studies have come to similar conclusions. Thus, it has been shown that isopropylnoradrenaline contrary to glucose does not stimulate glucose oxidation in isolated islets (Herrmann and Deckert, 1977), and that the insulin response to isopropylnoradrenaline is largely retained in insulin independent diabetics, despite a clear reduction of glucose stimulated insulin release among these patients (Robertson and Porte, 1973). Moreover, compared to normal NMRI-mice, mice belonging to a different strain displayed a markedly reduced insulin response to glucose, although they had a normal insulin secretory response to isopropylnoradrenaline (Lundquist et al., 1979). Also, vinblastine has been found to abolish the insulin secretory response to glucose but not to isopropylnoradrenaline (Malaisse et al., 1971; Ericson and Lundquist, 1975).

Other results, however, e.g. the reduction of glucose-induced insulin release by β-blockade (Cerasi, Efendić and Luft, 1969; cf. Figs. 3, 5, 6) and the potentiating synergism between isopropylnoradrenaline and glucose (Taborsky et al., 1979), suggest an important interaction between secretory pathways stimulated by glucose and β-adrenergic agonists, respectively. The nucleotide cyclic AMP has been suggested to be involved in the stimulus-secretion coupling initiated by both glucose and β-adrenergic agonists, and is perhaps a common link between these two insulin secretory pathways (Kuo, Hodgins and Kuo, 1973; Grill and Cerasi, 1974; Lefkowitz, 1975; Smith and Porte, 1976). Cyclic AMP has been suggested to exert a modulative effect on glucose-induced insulin secretion (cf. Hedeskov, 1980). This effect might in turn be dependent on intact β-adrenoceptors.

In conclusion, stimulation of the β-adrenergic receptors initiated a response leading to discharge of insulin; the mechanisms of this discharge seemed to be partially separate from that to glucose or to cholinergic stimulation. The selective β_2-agonist terbutaline was a far more potent insulin secretagogue than the selective β_1-agonist ITP. In addition, glucose-induced insulin secretion seemed dependent on intact β-adrenoceptors, but not to the same degree as the insulin response to terbutaline. Subdivision of the β-adrenoceptors regulating insulin secretion *in vivo* into β_1- and β_2-adrenoceptors seems to be less satisfactory, although they show similarities to the conventional β_2-adrenoceptors.

ACKNOWLEDGEMENTS

The authors express their appreciation to Åsa Amilon, Elsy Andersson, Lena Kvist and Peter Okmark för technical assistance and to Eva Björkbom for secretarial help. This study was supported by the Swedish Medical Research Council (04P-4289, 14X-4289) and by grants from the Medical Faculty, University of Lund, Sweden and Nordisk Insulinfond, Gentofte, Denmark.

REFERENCES

Åblad, B., K.O. Borg, E. Carlsson, L. Ek, G. Johnsson, T. Malmfors and C.-G. Regårdh, 1975, A survey of the pharmacological properties of metoprolol in animals and man, Acta Pharmacol. Toxicol. 36, suppl. V, 7.

Bergman, J., H. Persson and K. Wetterlin, 1969, Two new groups of selective stimulants of adrenergic ß-receptors, Experientia 25, 899.

Bilski, A., S. Dorries, J.D. Fitzgerald, R. Jessup, H. Tucker and J. Wale, 1979, ICI 118,551, a potent $ß_2$ adrenoceptor antagonist, Proc. Brit. Pharmacol. Soc. 17-19 Dec 1979, p. 29.

Black, J.W., W.A.M. Duncan and R.G. Shanks, 1965, Comparison of some properties of pronethalol and propranolol, Brit. J. Pharmacol. 25, 577.

Bruss, M.L. and A.L. Black, 1978, Enzymatic microdetermination of glycogen, Anal. Biochem. 84, 309.

Burns, J.J. and L. Lemburger, 1965, N-Tertiary butyl methoxamine, a specific antagonist of the metabolic actions of epinephrine, Fed. Proc. 24, 298.

Carruthers, S.G., J.P. Hosler, P. Pentikainen and D.L. Azarnoff, 1978, Pamatolol: Phase I evaluation of the pharmacodynamics of a cardioselective beta adrenoceptor blocking drug, Clin. Pharmacol. Ther. 24, 168.

Cerasi, E., S. Efendić and R. Luft, 1969, Role of adrenergic receptors in glucose-induced insulin secretion in man, Lancet 2, 301.

Coore, H.G. and P.J. Randle, 1964, Regulation of insulin secretion studied with pieces of rabbit pancreas incubated *in vitro*, Biochem. J. 93, 66.

Ericson, L.E. and I. Lundquist, 1975, Effect of vinblastine *in vivo* on ultrastructure and insulin releasing capacity of the B-cell following sulphonylurea and isopropyl-noradrenaline, Diabetologia 11, 467.

Furchgott, R.F., 1972, The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory, In: Handbook of Exp. Pharmacol. vol. 33: Catecholamines, eds. H. Blaschko and E. Muscholl (Springer-Verlag, Berlin, Heidelberg, New York) p. 283.

Furman, B.L. and F.M. Tayo, 1974, Effect of some ß-adrenoceptor blocking drugs on insulin secretion in the rat, J. Pharm. Pharmacol. 26, 512.

Grill, V. and E. Cerasi, 1974, Stimulation by D-glucose of cyclic adenosine 3':5'-monophosphate accumulation and insulin release in isolated pancreatic islets of the rat, J. Biol. Chem. 249, 4196.

Hedeskov, C.J., 1980, Mechanism of glucose-induced insulin secretion, Physiol. Rev. 60, 442.

Heding, L., 1966, A simplified insulin radioimmunoassay method, In: Labelled proteins in tracer studies, eds. L. Donato, G. Milhaud and J. Sirchis (Euratom, Brussel) p. 345.

Herrmann, L. Sparre and T. Deckert, 1977, The effect of epinephrine and isoproterenol on insulin secretion and glucose utilization in isolated islets of Langerhans from mice, Acta Endocrinol. (Kbh) 84, 105.

Howe, R. and R.G. Shanks, 1966, Problems in identification of the beta-adrenergic receptor, Physiol. Rev. 56, 317.

Irie, Y., T. Igawa, T. Hosokawa and Y. Saitoh, 1979, Alterations in blood levels of carbohydrate and lipid metabolites and of cyclic AMP mediated by β_1- and β_2-adrenoceptors in beagle dogs: Effects of procaterol, a new selective β_2-adrenoceptor agonist, Europ. J. Pharmacol. 53, 351.

Jenkinson, D.H., 1973, Classification and properties of peripheral adrenergic receptors, Brit. Med. Bull. 29, 142.

Johansson, B., 1979, Effects of atenolol, metoprolol, and pamatolol on cardiac and vascular beta-adrenoceptors in the rat, J. Cardiovasc. Pharmacol. 1, 287.

Kaneto, A., E. Miki and K. Kosaka, 1975, Effect of beta and beta$_2$ adrenoceptor stimulants infused intrapancreatically on glucagon and insulin secretion, Endocrinology 97, 1166.

Kuo, W.-N., D.S. Hodgins and J.F. Kuo, 1973, Adenylate cyclase in islets of Langerhans: Isolation of islets and regulation of adenylate cyclase activity by various hormones and drugs. J. Biol. Chem. 248, 2705.

Lands, A.M., A. Arnold, J.P. McAuliff, F.P. Luduena and T.G. Brown Jr., 1967, Differentiation of receptor systems activated by sympathomimetic amines, Nature 214, 597.

Lefkowitz, R.J., 1975, Heterogeneity of adenylate cyclase-coupled ß-adrenergic receptors, Biochem. Pharmacol. 24, 583.

Lenz, S., C. Kühl, P. Wang, L. Mølsted-Pedersen, H. Ørskov and O.K. Faber, 1979, The effect of ritodrine on carbohydrate and lipid metabolism in normal and diabetic pregnant women, Acta Endocrinol. (Kbh) 92, 669.

Loubatières, A., M.M. Mariani, G. Sorel and L. Savi, 1971, The action of ß-adrenergic blocking and stimulating agents on insulin secretion. Characterization of the type of ß receptor, Diabetologia 7, 127.

Loubatières-Mariani, M.M., J. Chapal, G. Ribes and A.-L. Loubatières, 1977, Discrepancies in the response of the insulin secreting cells of the dog and rat to different adrenergic stimulating agents, Acta Diabetol. Lat. 14, 144.

Lundquist, I., 1972a, Interaction of amines and aminergic blocking agents with blood glucose regulation. I. ß-Adrenergic blockade, Europ. J. Pharmacol. 18, 213.

Lundquist, I., 1972b, Interaction of amines and aminergic blocking agents with blood glucose regulation. II. α-Adrenergic blockade, Europ. J. Pharmacol. 18, 225.

Lundquist, I. and L.E. Ericson, 1978, ß-Adrenergic insulin release and adrenergic innervation of mouse pancreatic islets, Cell Tiss. Res. 193, 73.

Lundquist, I., R. Håkanson, J.B. Harris, R. Libelius and F. Sundler, 1979, Endocrine pancreas in the dystrophic mouse. In: Ann. New York Acad. Sci. vol. 317, ed. J.B. Harris (N.Y. Acad. Sci., New York) p. 206.

Malaisse, W., F. Malaisse-Lagae, P.H. Wright and J. Ashmore, 1967, Effects of adrenergic and cholinergic agents upon insulin secretion in vitro, Endocrinology, 80, 975.

Malaisse, W.J., F. Malaisse-Lage, M.O. Walker, P.E. Lacy, 1971, The stimulus-secretion coupling of glucose-induced insulin release. V. The participation of a microtubular-microfilamentous system, Diabetes 20, 257.

Omini, C., A.R. Sommerville, G.C. Folco, G. Rossoni and L. Pughsi, 1979, Interaction between β_1- and β_2-adrenoceptors in the isolated guinea pig trachea, Pharmacol. Res. Commun. 11, 529.

Porte Jr, D., A.L. Graber, T. Kuzuya and R.H. Williams, 1966, The effect of epinephrine on immunoreactive insulin levels in man, J. Clin, Invest. 45, 228.
Porte Jr, D., 1967a, A receptor mechanism for the inhibition of insulin release by epinephrine in man, J. Clin. Invest. 46, 86.
Porte Jr, D., 1967b, Beta adrenergic stimulation of insulin release in man, Diabetes, 16, 150.
Porte Jr, D., 1969, Sympathetic regulation of insulin secretion, Arch. Intern. Med. 123, 252.
Rerup, C. and I. Lundquist, 1966, Blood glucose level in mice. I. Evaluation of a new technique of multiple serial sampling, Acta Endocrinol. (Kbh) 52, 357.
Robertson, R.P. and D. Porte Jr, 1973, Adrenergic modulation of basal insulin secretion in man, Diabetes 22, 1.
Roszkowski, A.P., A.M. Strosberg, L.M. Miller, J.A. Edwards, B. Berkoz, G.S. Lewis, O. Halpern and J.H. Fried, 1972, Selective ß-adrenergic myocardial stimulant, Experientia, 28, 1336.
Smith, P.H. and D. Porte Jr, 1976, Neuropharmacology of the pancreatic islets, Ann. Rev. Pharm. Tox. 16, 269.
Taborsky Jr., G.J., P.H. Smith, J.B. Halter and D. Porte Jr., 1979, Glucose infusion potentiates the acute insulin response to non-glucose stimuli during the infusion of somatostatin, Endocrinology 105, 1215.
Wilkenfeld, B.E. and B. Levy, 1968, Adrenergic blocking properties of MJ 1999 and butoxamine on cardiac and vascular beta-receptors, Arch. Int. Pharmacodyn. Ther. 176, 218.

Effects of Autonomic Blockade by Methylatropine and Optical Isomers of Propranolol on Plasma Insulin Levels in the Basal State and after Stimulation

Bo Ahrén and Ingmar Lundquist

Department of Pharmacology, University of Lund, Lund, Sweden

SUMMARY

The effects of autonomic blockade on plasma concentrations of immunoreactive insulin in the basal state and after stimulation with four different secretagogues were investigated *in vivo* in conscious mice.

The muscarinic blocker methylatropine slightly depressed basal insulin, and almost totally abolished the insulin response to the cholinergic agonist carbachol, whereas the insulin response to glucose and glucagon was unaffected. Contrary to these findings, the insulin response to the β_2-adrenoceptor agonist terbutaline was potentiated, by about 75%.

The β-adrenoceptor blocker L-propranolol depressed basal insulin by about 60%, and totally abolished the insulin responses to glucagon and terbutaline. The insulin response to glucose was slightly reduced and that to carbachol was unaffected by L-propranolol. The stereoisomer D-propranolol which is devoid of β-adrenoceptor blocking activity but exerts local anaesthetic effect of the same potency as the L-isomer, was without effect on basal insulin levels and the insulin responses to the different secretagogues.

It is concluded that basal concentrations of immunoreactive insulin is substantially dependent on intact β-adrenoceptors and more moderately on intact muscarinic receptors. The insulin responses to terbutaline and glucagon are closely connected to the β-adrenoceptors, and to carbachol to the muscarinic receptors. Glucose-induced insulin response seems largely unaffected by autonomic receptor blockade although a slight reduction after β-adrenoceptor blockade is demonstrable.

INTRODUCTION

The pancreatic islets are innervated by parasympathetic as well as sympathetic nerves and the influences of this autonomic innervation on basal and stimulated insulin secretion have previously been investigated (cf.

Woods and Porte 1974, Gerich and Lorenzi 1978, Edwards and Bloom 1978). Thus, electrical vagal stimulation (Frohman et al. 1967, Kaneto et al. 1967) and cholinergic agonists (Kaneto et al. 1968, Iversen 1973, Kaneto et al. 1974) initiate release of insulin and these effects can be blocked by the muscarinic blocker atropine (Findlay et al. 1969, Kaneto and Kosaka 1974). On the other hand, atropine has been reported to be without effect on glucose-induced insulin response in man (Hendersen et al. 1976) and on basal insulin levels in the dog (Porte et al. 1973) and the calf (Bloom et al. 1974).

The sympathetic nervous system inhibits insulin secretion which has been demonstrated after direct electrical stimulation of the splanchnic nerves (Porte et al. 1973, Bloom and Edwards 1975, Miller 1975) and indirect activation of the sympatho-adrenal system (Järhult and Holst 1978, Järhult and Holst 1979). α-Adrenoceptor blockade by phentolamine increases basal insulin in several species (Frohman et al. 1967, Werrbach et al. 1970, Hertelendy et al. 1970, Järhult et al. 1979), and α-adrenoceptor stimulation by adrenaline and noradrenaline inhibit insulin secretion (Porte and Williams 1966, Porte et al. 1966). Thus, it seems as if the inhibitory effect of the sympathetic system is mediated by α-adrenoceptors. In addition, since the insulin cell is equipped with stereoselective ß-adrenoceptors (Lundquist and Ericson 1978) and stimulation of these receptors promote insulin release (Porte 1967) the sympathetic nervous system has the potential ability to exert a dual effect on insulin release; the stimulatory effect being exerted through ß-adrenoceptor activation. This is corroborated by the finding that in the presence of phentolamine a splanchnic nerve stimulation produces an enhanced insulin secretion (Miller 1975).

Furthermore, ß-adrenoceptor blockade by propranolol has been reported to depress basal insulin levels in several species (Gagliardino et al. 1970, Werrbach et al. 1970, Lundquist 1972). It has also been reported that propranolol inhibits the insulin response to glucose (Cerasi et al. 1972, Furman and Tayo 1974) but a recent study has proposed that this might be due to the local anaesthetic property of the drug (Myers and Hope-Gill 1979).

In this study the effects of autonomic blockade by methylatropine and propranolol on basal insulin levels and on stimulated insulin responses were investigated *in vivo* in conscious mice. Four different insulin secretagogoues were selected to elevate plasma insulin concentrations; these were glucose, the cholinergic agonist carbachol, the β_2-adrenoceptor agonist terbutaline, and glucagon; these agents probably display their insulin releasing effects through at least partially different mechanisms of action.

MATERIAL AND METHODS

Animals. Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark), weighing 25-35 g, were used throughout the experiments. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden) and tap water *ad libitum* before and during the experiments.

Drugs. D-glucose and carbachol were from British Drug Houses Ltd, Poole, England, terbutaline (1-(3,5-dihydroxyphenyl)-2-(*tert*-butylamino)ethanol sulfate) from Draco AB, Lund, Sweden, methylatropine from Vitrum AB, Stockholm, Sweden and D- and L-propranolol (3-(1-naphtyloxy)-2-propanol-1-isopropylamino hydrochloride) from ICI, Macclesfield, England. These drugs were dissolved in 0.154 mol/l saline. Pure porcine glucagon was from Novo Research Institute, Copenhagen, Denmark, and it was dissolved in 0.154 mol/l

saline with addition of 1 g/l gelatine to avoid adsorbtion.

Experiments. Methylatropine, 8.2 µmol/kg, D-propranolol, 9.6 µmol/kg, L-propranolol, 9.6 µmol/kg, or saline was injected intraperitoneally, 10 µl/g body weight was given. Twenty min later D-glucose, 2.8 mmol/kg, carbachol 0.16 µmol/kg, terbutaline, 3.6 µmol/kg, glucagon, 4.3 nmol/kg or saline, was injected intravenously; 10 µl/g body weight was given. Blood samples were taken by orbital puncture 2 (D-glucose, carbachol or glucagon) or 5.5 (terbutaline) min after the last injection. In initial experiments it was found that the peak level of plasma concentrations of immunoreactive insulin is achieved about 2 (D-glucose, carbachol or glucagon) or 5.5 (terbutaline) min after an intravenous injection. The various pretreatments did not alter this duration. In one series of experiments carbachol, 0.16 µmol/kg or 4.0 µmol/kg, was given intraperitoneally to mice pretreated with saline or L-propranolol (9.6 µmol/kg or 36 µmol/kg) and blood samples were taken 5 min after the i.p. injection of carbachol. The doses chosen were half-maximal with regard to the increase in plasma concentrations of immunoreactive insulin after the intravenous injection for all agents except glucagon, which was injected in a dose yielding an insulin secretion comparable to that of the other agents.

Determination of Insulin and Glucose. The concentration of immunoreactive insulin (IRI) in plasma was determined by radioimmunoassay (Heding 1966), using ^{125}I-labelled pig insulin and guinea pig anti-pig insulin. The plasma glucose concentration was determined by a glucose oxidase method (Bruss and Black 1978).

Statistics. Means and standard errors of the mean are given. Student's t-test was employed for tests of significance.

RESULTS

Basal Plasma IRI. Fig. 1 shows the effects of methylatropine and L- and D-propranolol on basal plasma IRI. It is seen that methylatropine slightly (by about 10%; $p < 0.05$) and L-propranolol markedly (by about 60%; $p < 0.001$) depressed basal insulin, whereas D-propranolol was without effect. Plasma glucose was 9.8 ± 0.2 mmol/l in the control group. There were no differences with regard to plasma glucose amongst the groups.

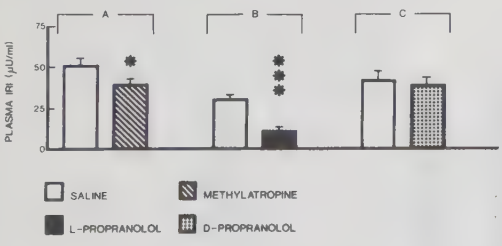


Fig. 1

Plasma concentrations of immunoreactive insulin 2 min after i.v. injection of saline (0.154 mol/l). Methylatropine (8.2 µmol/kg; Fig. 1A), L-propranolol (9.6 µmol/kg; Fig. 1B) or D-propranolol (9.6 µmol/kg; Fig. 1C) was injected i.p. 20 min before the i.v. injection. Controls were given saline i.p. There were 33-36 animals in the methylatropine-treated group and its control and there were 17-19 animals in the groups treated with L- and D-propranolol and their controls. Bars indicate SEMs. Asterisks indicate probability level of random difference. * $p < 0.05$, *** $p < 0.001$.

Glucose-Induced Insulin Response. Fig. 2 shows that glucose injection increased the plasma IRI concentrations by about 90 µU/ml. Neither methylatropine nor D-propranolol influenced this response to glucose. However, L-propranolol inhibited the net insulin response to glucose by about 20% ($p < 0.05$) taking into account the decreased basal plasma insulin levels

after L-propranolol. Plasma glucose concentrations were 16.2 ± 0.4 mmol/l in the glucose-treated control group. There was no difference with regard to plasma glucose amongst the glucose-treated groups. The saline injected control group had a plasma glucose concentration of 9.1 ± 0.1 mmol/l.

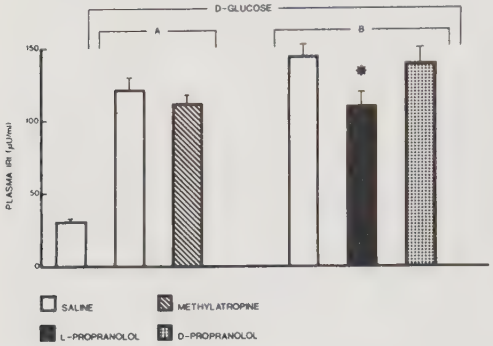


Fig. 2

Plasma concentrations of immunoreactive insulin (peak level) after the i.v. injection of D-glucose (2.8 mmol/kg). Saline, methylatropine (8.2 µmol/kg; Fig. 2A), L- or D-propranolol (9.6 µmol/kg; Fig. 2B) was injected i.p. 20 min before the i.v. injection of glucose. Controls were injected with saline i.p. as well as i.v. There were 15-26 animals in each group, except in the methylatropine-treated group, which consisted of 8 animals. Bars indicate SEMs. Asterisk indicates probability level of random difference. * $p < 0.05$.

Terbutaline-Induced Insulin Response. Terbutaline injection enhanced plasma insulin by about 90 µU/ml. In Fig. 3 it can be seen that L-propranolol totally abolished this insulin response to terbutaline. In fact, it seemed as if L-propranolol in these animals depressed basal insulin even more than in control animals (cf. Fig. 1). D-propranolol had no effect on terbutaline-induced insulin response, but methylatropine increased the response to terbutaline; it was potentiated by about 75% ($p < 0.001$). Plasma glucose concentrations were 11.6 ± 0.6 mmol/l in the terbutaline-treated control group; there was no difference in plasma glucose amongst the groups. The saline injected control group had a plasma glucose concentration of 11.0 ± 0.4 mmol/l. Thus, terbutaline did not induce any change in plasma glucose during the time-period studied.

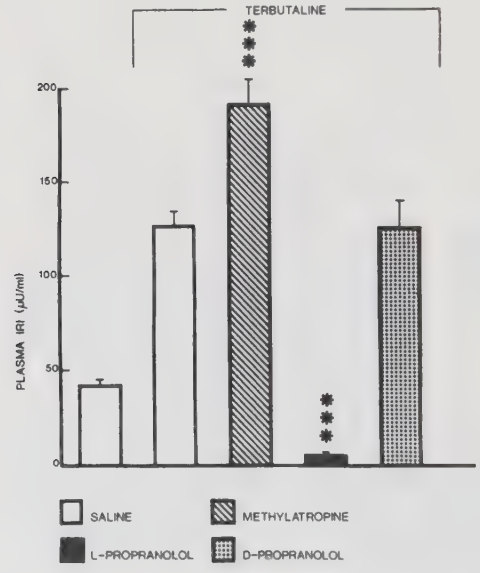


Fig. 3

Plasma concentrations of immunoeactive insulin (peak level) after the i.v. injection of terbutaline (3.6 µmol/kg). Saline, methylatropine (8.2 µmol/kg), L- or D-propranolol (9.6 µmol/kg) was injected i.p. 20 min before the i.v. injection of terbutaline. Controls were injected with saline i.p. as well as i.v. There were 16-26 animals in each group. Bars indicate SEMs. Asterisks indicate probability level of random difference. *** $p < 0.001$.

Glucagon-Induced Insulin Response. Injection of glucagon induced an elevation of plasma IRI to about 75 μU/ml. Fig. 4 shows that L-propranolol totally abolished this insulin response, whereas D-propranolol and methylatropine was without effect. Plasma glucose concentrations were 8.5 ± 0.2 mmol/l in the glucagon-treated control group; there were no differences in plasma glucose amongst the groups. The control group had a plasma glucose concentration of 8.7 ± 0.2 mmol/l. Thus, no changes in plasma glucose levels were observed during the time period studied.

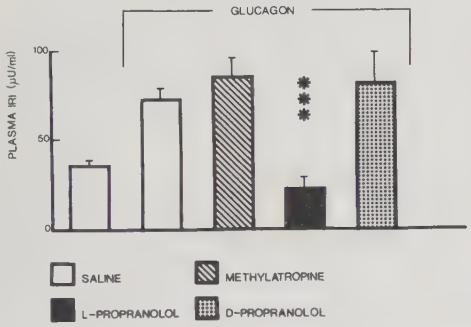


Fig. 4

Plasma concentrations of immunoreactive insulin (peak level) after the i.v. injection of glucagon (4.3 nmol/kg). Saline, methylatropine (8.2 μmol/kg), L- or D-propranolol (9.6 μmol/kg) was injected i.p. 20 min before the i.v. injection of glucagon. Controls were injected with saline i.p. as well as i.v. There were 7-15 animals in each group. Bars indicate SEMs. Asterisks indicate probability level of random difference. *** $p < 0.001$.

Carbachol-Induced Insulin Response. The i.v. injection of carbachol elevated the plasma IRI concentrations to about 150 μU/ml. Methylatropine totally abolished this response and D-propranolol was without effect (Fig. 5). From the experiments with the combined treatment of L-propranolol and i.v. injection of carbachol it was difficult to draw any conclusions because of the severe, though transient, circulatory derangement that evolved. Instead carbachol was injected i.p. after the treatment with L-propranolol or saline, and it was concluded (Fig. 5) that L-propranolol had no influence on the comparable slight, elevation of plasma IRI in response to carbachol. Neither a greater insulin response induced by carbachol was influenced by L-propranolol. Plasma glucose levels were 9.0 ± 0.2 mmol/l in control mice treated with carbachol i.v., and 8.7 ± 0.3 mmol/l in those treated with carbachol i.p. Saline-injected control mice had plasma glucose concentrations of 8.6 ± 0.2 mmol/l. Pretreatment with methylatropine or the two isomers of propranolol did not alter these plasma glucose concentrations during the time-period studied.

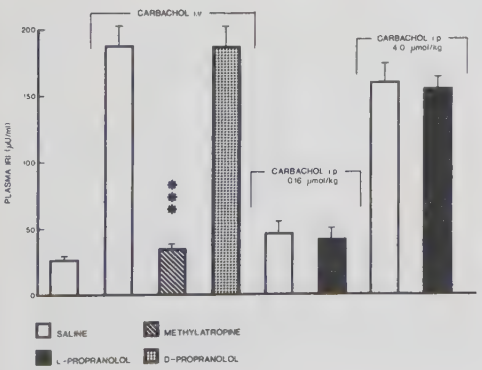


Fig. 5

Plasma concentrations of immunoreactive insulin (peak level) after the i.v. injection of carbachol (0.16 μmol/kg) (left panel) or after the i.p. injection of saline or carbachol (0.16 μmol/kg or 4.0 μmol/kg) (right panel). Saline, methylatropine (8.2 μmol/kg), L-propranolol (9.6 μmol/kg together with 0.16 μmol/kg carbachol and 36 μmol/kg together with 4.0 μmol/kg carbachol) or D-propranolol (9.6 μmol/kg) was injected i.p. 20 min before the carbachol injection. Controls were injected with saline i.p. as well as as i.v. There were 10-25 animals in each group. Bars indicate SEMs. Asterisks indicate probability level of random difference. *** $p < 0.001$.

DISCUSSION

An important role for basal plasma insulin concentrations is played by plasma glucose but hormonal, neural and metabolic influences are also of great importance *in vivo* (cf. Turner et al. 1980). This study shows that cholinergic blockade by methylatropine and β-adrenoceptor blockade by L-propranolol markedly depress basal insulin *in vivo* in conscious mice. Since D-propranolol, which exerts a local anaesthetic effect of about the same potency as L-propranolol (Barrett and Cullum 1968) but is without β-adrenoceptor blocking effect (Howe and Shanks 1966), did not affect basal insulin, the recorded depression effect of plasma insulin seen after L-propranolol can be ascribed to the β-adrenoceptor blocking effect rather than to the membrane stabilizing effect. This indicates that intact muscarinic receptors are of some and β-adrenergic receptors of substantial importance for basal insulin. A decrease in basal insulin after propranolol was earlier reported in man (Gagliardino et al. 1970), dog (Werrbach et al. 1970) and mouse (Lundquist 1972), whereas atropine earlier has been shown to be without effect on basal insulin in man (Henderson et al. 1976) and calves (Bloom et al. 1974). Species or dose differences might be a reason for the discrepancy with the present study concerning the effect of muscarinic blockade. Moreover, the use of methylatropine eliminates central effects which might add to the peripheral effects in studies where atropine is used.

Glucose, the β_2-adrenoceptor stimulator terbutaline, the polypeptide glucagon, and the cholinergic agonist carbachol elevated plasma IRI concentrations. Some of these agents might influence peripheral blood flow, but since pancreatic blood flow seems not to be a critical factor for insulin secretion (Rappaport et al. 1971) it is reasonable to assume that the effects on plasma IRI by them are unrelated to their vasoeffects and is a reflection of a true response in insulin.

L-propranolol in contrast to D-propranolol, inhibited the insulin response to glucagon, to the β_2-adrenoceptor agonist terbutaline, and to glucose. Again, it can be concluded that the local anaesthetic property of propranolol is not responsible for these effects, since D-propranolol did not influence the responses. Thus, our study in the mouse confirms those made in man (Cerasi et al. 1972) and rat (Furman and Tayo 1974) that glucose-induced insulin release is partially dependent on intact β-adrenoceptors.

Methylatropine, the muscarinic blocker, inhibited as expected the insulin response to cholinergic stimulation with carbachol. It had no effect on the insulin response to glucose and glucagon, and thus these secretagogues seem to be independent of cholinergic receptors for their effects. Surprisingly, pretreatment with methylatropine was followed by a potentiated insulin response to terbutaline. It might be speculated that cholinergic blockade leads to a diminished formation of cyclic GMP which subsequently is followed by an enhanced responsiveness of the adenylate cyclase--cAMP system which is closely linked to β-adrenoceptor effects (cf. George et al. 1970, Illiano et al. 1973; Kuo et al. 1973). Available evidence regarding the effect of cholinergic stimulation on islet guanylate cyclase--cGMP system is contradictory, regarding activation (Howell and Montague 1974) or no effect (Gagerman et al. 1978).

It is concluded from the results of the present study that basal plasma insulin *in vivo* in conscious mice is dependent on both β-adrenoceptors and muscarinic receptors, and that L-propranolol and methylatropine influence stimulated insulin response to different agents with effects dependent on the nature of the secretagogues. Terbutaline, glucagon and glucose seem to initiate events more or less sensitive to β-adrenoceptor blockade in their ability to enhance plasma IRI, whereas cholinergic sti-

mulation is totally independent of such events. Moreover, glucose and glucagon exert their effects despite muscarinic blockade, while insulin response to β_2-adrenoceptor stimulation by terbutaline is enhanced after cholinergic blockade.

ACKNOWLEDGEMENTS

The authors express their appreciation to Lena Kvist and Peter Okmark for technical assistance and to Eva Björkbom for secretarial help. This study was supported by the Swedish Medical Research Council (14P-4289, 14X-4286) and by grants from the Medical Faculty, University of Lund, Sweden and Nordisk Insulinfond, Gentofte, Denmark.

REFERENCES

BARRETT, A.M. & CULLUM, V.A. 1968. The biological properties of the optical isomers of propranolol and their effects on cardiac arrythmias. Brit J Pharmacol 34:43-55.

BLOOM, S.R. & EDWARDS, A.V. 1975. The release of pancreatic glucagon and inhibition of insulin in response to stimulation of the sympathetic innervation. J Physiol 253:157-173.

BLOOM, S.R., EDWARDS, A.V. & VAUGHAN, N.J.A. 1974. The role of the autonomic innervation in the control of glucagon release during hypoglycemia in the calf. J Physiol 236:611-623.

BRUSS, M.L. & BLACK, A.L. 1978. Enzymatic microdetermination of glycogen. Analyt Biochem 84:309-312.

CERASI, E., LUFT, R. & EFENDIĆ, S. 1972. Effect of adrenergic blocking agents on insulin response to glucose infusion in man. Acta Endocrinol (Kbh) 69: 335-346.

EDWARDS, A.V. & BLOOM, S.R. 1978. Nervous control of pancreatic hormones. In: BLOOM, S.R. (ed.) Gut hormones. Churchill Livingstone, Edinburgh. pp 394-405.

FINDLAY, J., GILL, J., LEVER, J., RANDLE, P. & SPRIGGS, T. 1969. Increased insulin output following stimulation of the vagal supply to the perfused rabbit pancreas. J Anat 104:580-584.

FURMAN, B. & TAYO, F. 1974. Effect of some ß-adrenoceptor blocking drugs on insulin secretion in the rat. J Pharm Pharmacol 26:512-517.

FROHMAN, L., EZDINK, E. & JAVID, R. 1967. Effect of vagotomy and vagal stimulation on insulin secretion. Diabetes 16:443-448.

GAGERMAN, E., IDAHL, L.-Å., MEISSNER, H.P. & TÄLJEDAL, I.-B. 1978. Insulin release, cGMP, cAMP, and membrane potential in acetylcholine-stimulated islets. Am J Physiol 235:E 493-E 500.

GAGLIARDINO, J., BELLONE, C., DORIA, I., SANCHEZ, J. & PEREYRA, V. 1970. Adrenergic regulation of basal serum glucose, NEFA, and insulin levels. Horm Metab Res 2:318-322.

GEORGE, W., POLSON, J., O'TOOLE, A. & GOLDBERG, N. 1970. Elevation of guanosine 3',5'cyclic phosphate in rat heart after perfusion with acetyl choline. Proc Natl Acad Sci USA 66:398-403.

GERICH, J.E. & LORENZI, M. 1978. The role of the autonomic nervous system and somatostatin in the control of insulin and glucagon secretion. In:

GANONG, W.F. & MARTINI, L. (eds). Frontiers in Neuroendocrinology, vol. 5. Raven Press, New York. pp 265-288.

HEDING, L. 1966. A simplified insulin radioimmunoassay method. In: DONATO, L., MILHAUD, G. & SIRCHIS, J. (eds). Labelled proteins in tracer studies. Euratom, Brussels. pp 345-350.

HENDERSON, J., JEFFERYS, D., JONES, R. & STANLEY, D. 1976. The effect of atropine on the insulin release caused by oral and intravenous glucose in human subjects. Acta Endocrinol (Kbh) 83:772-780.

HERTELENDY, F., TAKAHASHI, K., MACHLIN, L.J. & KIPNIS, D.M. 1970. The effect of chronic adrenergic blockade on the inhibition by epinephrine of growth hormone and insulin release in sheep. Horm Metab Res 2:257-259.

HOWE, R. & SHANKS, R.G. 1966. Optical isomers of propranolol. Nature 210: 1336-1338.

HOWELL, S.L. & MONTAGUE, W. 1974. Regulation of guanylate cyclase in guinea-pig islets of Langerhans. Biochem J. 142:379-384.

ILLIANO, G., TELL, P., SIEGEL, M. & CUATRECASAS, P. 1973. Guanosine 3',5'-cyclic monophosphate and the action of insulin and acetylcholine. Proc Natl Acad Sci USA 70:2443-2447.

IVERSEN, J. 1973. Effect of acetylcholine on the secretion of glucagon and insulin from the isolated, perfused canine pancreas. Diabetes 22:381-387.

JÄRHULT, J., AHRÉN, B. & LUNDQUIST, I. 1979. Inhibitory effect of somatostatin on insulin secretion during α-adrenergic blockade in three different species. Acta Endocrinol (Kbh) 92:166-173.

JÄRHULT, J., & HOLST, J.J. 1978. Reflex adrenergic control of endocrine pancreas evoked by unloading of carotid baroreceptors in cats. Acta Physiol Scand 104:188-202.

JÄRHULT, J. & HOLST, J. 1979. The role of the adrenergic innervation to the pancreatic islets in the control of insulin release during exercise in man. Pflügers Arch 383:41-45.

KANETO, A. & KOSAKA, K. 1974. Stimulation of glucagon and insulin secretion by acetylcholine infused intrapancreatically. Endocrinology 95:676-681.

KANETO, A., KOSAKA, K. & NAKAO, K. 1967. Effects of stimulation of the vagus nerve on insulin secretion. Endocrinology 80:530-536.

KANETO, A., MIKI, E. & KOSAKA, K. 1974. Effects of vagal stimulation on glucagon and insulin secretion. Endocrinology 95:1005-1010.

KUO, W., HODGINS, D. & KUO, J. 1973. Adenylate cyclase in islets of Langerhans: Isolation of islets and regulation of adenylate cyclase activity by various hormones and drugs. J Biol Chem 248:2705-2711.

LUNDQUIST, I. 1971. Insulin secretion. Its regulation by monoamines and acid amyloglucosidase. Acta Physiol Scand. Suppl 372:1-47.

LUNDQUIST, I. 1972. Interaction of amines and aminergic blocking agents with blood glucose regulation. I. β-Adrenergic blockade. Europ J Pharmacol 18:213-224.

LUNDQUIST, I. & ERICSON, L.E. 1978. β-Adrenergic insulin release and adrenergic innervation of mouse pancreatic islets. Cell Tiss Res 193:73-85.

MILLER, R.E. 1975. Neural inhibition of insulin secretion from the isolated canine pancreas. Am J Physiol 229:144-149.

MYERS, M.G. & HOPE-GILL, H.F. 1979. Effect of D- and DL-propranolol on glucose-stimulated insulin release. Clin Pharmacol Therap 25:303-308.

PORTE, D. Jr. 1967. Beta adrenergic stimulation of insulin release in man. Diabetes 16:150-155.

PORTE, D. Jr, GIRARDIER, L., SEYDOUX, J., KANAZAWA, Y. & POSTERNAK, H. 1973. Neural regulation of insulin secretion in the dog. J Clin Invest 52:210-214.

PORTE, D. Jr, GRABER, A.L., KUZUYA, T. & WILLIAMS, R. H. 1966. The effect of epinephrine on immunoreactive insulin levels in man. J. Clin Invest 45:228-236.

PORTE, D. Jr & WILLIAMS, R.H. 1966. Inhibition of insulin release by norepinephrine in man. Science 152:1248-1250.

RAPPAPORT, A.M., KAWAMURA, T., DAVIDSON, J.K., LIN, B.J., OHIRA, S., ZEIGLER, M., CODDLING, J.A., HENDERSON, J. & HAIST, R.E. 1971. Effects of hormones and of blood flow on insulin output of isolated pancreas in situ. Am. J. Physiol. 221:343-348.

TURNER, R.C., LANG, D.A. & WARD, G.M. 1980. Control of basal insulin secretion. In: WALDHÄUSL, W (ed). Diabetes 1979. Excerpta Medica, Amsterdam. pp 544-549.

WOODS, S.C. & PORTE, D. Jr. 1974. Neural control of the endocrine pancreas. Physiol Rev 54:596-619.

Influence of the Sympatho-Adrenal System and Somatostatin on the Secretion of Insulin in the Rat

B. Ahrén, J. Järhult and I. Lundquist

Departments of Pharmacology, Physiology, and Surgery, University of Lund, Lund, Sweden

SUMMARY

1. The effects of somatostatin on insulin secretion in anaesthetized rats subjected to different manipulations of the sympatho-adrenal system have been investigated.

2. Somatostatin (0.1 μg/min) inhibited the secretion of insulin in intact rats both in the basal state and after inducing an enhanced insulin release by infusion of the α-adrenoceptor-blocker phentolamine.

3. Combined surgical splanchnicotomy and adrenalectomy caused an increase in the basal plasma insulin concentration. Somatostatin (0.1 μg/min) inhibited basal insulin release also in these rats. After infusion of phentolamine, however, the dose of somatostatin had to be raised fivefold (0.5 μg/min) to achieve a comparable inhibition of insulin release. On the other hand, a similar rate of insulin secretion induced by glucose in intact rats could be inhibited by the lower dose of somatostatin.

4. Administration of the β-adrenoceptor-blocking agent propranolol to splanchnicotomized-adrenalectomized rats lowered basal insulin secretion to the same level as seen in intact rats. In these β-adrenoceptor-blocked rats somatostatin (0.1 μg/min) inhibited insulin release both in the presence and absence of α-adrenoceptor blockade.

5. Rats subjected to chemical sympathectomy through pretreatment with 6-hydroxydopamine together with adrenalectomy displayed plasma insulin concentrations slightly above the normal range, but the values were much lower than in splanchnicotomized-adrenalectomized rats. Infusion of phentolamine to the chemically sympathectomized rats did not further increase insulin secretion, and somatostatin (0.1 μg/min) depressed insulin release both in the absence and presence of α-adrenoceptor blockade.

6. It is suggested that an inhibitory tone exerted by the splanchnic nerves modulates the basal insulin secretion in the rat. Somatostatin and the sympatho-adrenal system show a complex interaction on the insulin cells in that the sensitivity to somatostatin in splanchnicotomized-adrenalectomized rats with intact β-adrenoceptors is decreased in the presence of the α-adrenoceptor-blocker phentolamine. The exact mechanism behind this decreased sensitivity remains unclear.

INTRODUCTION

It has been demonstrated that adrenergic nerves innervate the islets of Langerhans and adrenergic nerve terminals have been demonstrated in contact with insulin cells (Esterhuizen, Spriggs & Lever, 1968; Lundquist & Ericson, 1978). It has also been shown that the sympatho-adrenal system, consisting of the adrenergic nervous system and hormones secreted from the adrenal medulla, can influence the release of insulin (cf. Smith & Porte, 1976; Gerich & Lorenzi, 1978). Thus, catecholamines may inhibit insulin release via stimulation of the α-adrenoceptors or augment insulin release via stimulation of the ß-adrenoceptors (Porte, 1967; cf. Lundquist, 1971). Furthermore, plasma insulin concentration is decreased in response to activation of the sympathetic nerves either directly by electrical stimulation of the splanchnic nerves (Porte, Girardier, Seydoux, Kanazawa & Posternak, 1973; Bloom & Edwards, 1975; Miller, 1975) or indirectly by unloading of the carotid baroreceptors (Järhult & Holst, 1978) or by exercise (Galbo, Christensen & Holst, 1977; Järhult & Holst, 1979).

The heptadecapeptide somatostatin has been demonstrated to occur in endocrine cells of the pancreatic islets and the gut (Hökfelt, Efendić, Hellerström, Johansson, Luft & Arimura, 1975). It is a powerful inhibitor of insulin secretion and may function as a hormone or by paracrine actions (cf. Luft, Efendić & Hökfelt, 1978). Somatostatin has been suggested to exert its effects on the insulin cell via stimulation of the inhibitory α-adrenoceptors in the dog (Smith, Woods & Porte, 1976). However, other evidence from several studies in different species has clearly demonstrated that stimulation of α-adrenoceptors is of little importance for the inhibitory effect of somatostatin on insulin release (Efendić & Luft, 1975; Kaneto, Kajinuma, Kaneko & Kosaka, 1978; Schmitt, Lorenzi, Gerich, Bohannon, Karam & Forsham, 1979; Järhult, Ahrén & Lundquist, 1979; Hara, Patton & Gerich, 1979), although the results of these studies cannot exclude the existence of a more complex interaction between somatostatin and the sympatho-adrenal system on the insulin cell.

The aim of the present investigation was to study in more detail the influences of the sympatho-adrenal system and somatostatin on insulin secretion in the anaesthetized rat.

METHODS

Animals. The experiments were carried out on adult Wistar rats (270-295 g b.w.) purchased from Møllegaard Avlslaboratorium, Skensved, Denmark. The animals were kept on standard pellets (Astra-Ewos, Södertälje, Sweden) and tap water ad libitum.

Drugs. Synthetic cyclic somatostatin (Beckman Ltd., Geneva, Switzerland) was dissolved in saline with the addition of 0.1% gelatine to avoid adsorbtion to tubes. Phentolamine (Regitin^{R}, CIBA-Geigy AG, Basel, Switzerland) was dissolved in saline. 6-Hydroxydopamine (AB Hässle, Mölndal, Sweden) was dissolved in ice-cold saline with the addition of 10 mg/ml ascorbic acid immediately before the injections. Propranolol (Inderal^{R}) was obtained from ICI Ltd., Macclesfield, England, D-glucose from British Drug Houses Ltd., Poole, England, sodium pentobarbital from ACO AB, Solna, Sweden and ascorbic acid from E. Merck AG, Darmstadt, FRG.

Surgical pretreatment. Under light ether anaesthesia the splanchnic nerves were identified via a midline incision. In one group of rats, the bilateral splanchnic nerves were cut just beneath the diaphragm and in another group the abdomen was closed without any interference with the sympatho-

-adrenal system. The experiments were performed two weeks later (see below).

Chemical sympathectomy. In one group of rats 6-hydroxydopamine, 65 mg/kg b.w., dissolved in ice-cold ascorbic acid (10 mg/ml) and saline, was injected in a tail vein. Control animals were injected with ascorbic acid dissolved in saline. The experiments were performed two days later (see below).

Experimental procedures. The rats were anaesthetized with sodium pentobarbital, 60 mg/kg b.w.. In rats pretreated with surgical splanchnicotomy or chemical sympathectomy, the abdomen was opened and the adrenal glands were removed. Polyethylene catheters were inserted into the right femoral vessels. The venous catheter was connected to a pump and used for infusion of drugs and the arterial catheter was used for collection of blood samples.

Protocol. After two control blood samples somatostatin was infused for 15 min at a dose of 0.1 µg/min. 15 min after the end of the somatostatin infusion, phentolamine (0.015 mg/min) or glucose (12.5 mg/min) was infused for 45 min. 15 min after the start of the infusion of phentolamine or glucose, somatostatin (0.1 µg/min or 0.5 µg/min) or saline was infused for a period of 15 min. All drugs were administered at a rate of 0.1 ml/min. In one group of rats propranolol (2 mg) was given i.p. 15 min before and 20 min after the start of the first somatostatin infusion.

The periods of the different infusions and the intervals between the blood samples are seen in the figures.

Determination of insulin and glucose. Plasma immunoreactive insulin concentrations (IRI) were measured by radioimmunoassay (Heding, 1966) using ^{125}I-labelled pig insulin and guinea pig anti-pig insulin. Plasma glucose concentrations were measured with a glucose oxidase method (Bruss & Black, 1978).

Statistics. All data are presented as mean values $\pm$ s.e.m. Student's t-test or t-test for paired observations was employed for the statistical evaluations.

RESULTS

Anaesthetized rats were subjected to different manipulations with the sympatho-adrenal system, and fig. 1 shows the basal plasma insulin concentrations in response to these manipulations. Mean basal IRI levels were slightly elevated in rats subjected to chemical sympathectomy by 6-hydroxydopamine plus adrenalectomy and markedly higher in rats subjected to splanchnic denervation and adrenalectomy (= splanchnicotomized-adrenalectomized) compared to intact rats (= sham operated). No difference was observed between splanchnicotomized-adrenalectomized rats given β-blockade and intact rats. Further, no difference in basal plasma glucose levels was observed between the different groups.

The next series of experiments was designed in order to compare the effects of somatostatin and phentolamine on plasma IRI concentrations in intact rats and in splanchnicotomized-adrenalectomized rats. Fig. 2 illustrates the changes in mean arterial plasma IRI and glucose concentrations in response to somatostatin (0.1 µg/min) before and during infusion of the α-adrenoceptor blocker phentolamine in intact rats and fig. 3 shows the effects of somatostatin (0.1 µg/min) before and during infusion of phentolamine in splanchnicotomized-adrenalectomized rats. Mean basal plasma

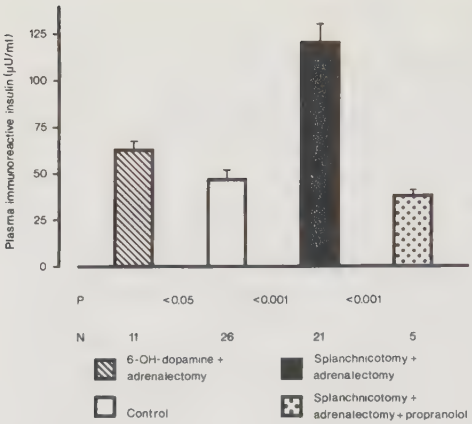
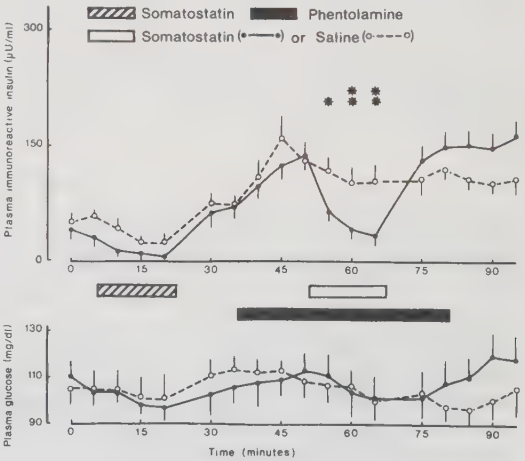


Fig. 1
Basal arterial plasma concentrations of immunoreactive insulin in rats exposed to different sympatho-adrenal manipulations. Rats were either pretreated with 6-hydroxydopamine plus adrenalectomy, surgical splanchnicotomy plus adrenalectomy or surgical splanchnicotomy plus adrenalectomy together with injection of propranolol. P = probability level of random difference. N = number of animals. Means and s.e.m. are given.

IRI concentration was 42.8 $\pm$ 7.6 µU/ml in intact rats and 115.6 $\pm$ 11.2 µU/ml in splanchnicotomized-adrenalectomized rats, respectively ($p < 0.001$). Somatostatin decreased basal plasma IRI concentration by about 75% in both intact and splanchnicotomized-adrenalectomized rats ($p < 0.001$) and infusion of phentolamine (0.015 mg/min) invariably increased their plasma IRI concentrations. However, infusion of somatostatin (0.1 µg/min) during the α-adrenoceptor blockade inhibited insulin secretion in intact rats but not in splanchnicotomized-adrenalectomized rats. Splanchnicotomized-adrenalectomized rats thus displayed a decreased sensitivity to somatostatin during α-adrenoceptor blockade.

Fig. 2
Arterial plasma concentrations of immunoreactive insulin and glucose in two groups of intact rats. Somatostatin (0.1 µg/min) was infused for 15 min in the basal state in both groups. During a subsequent administration of phentolamine (0.015 mg/min), an infusion of somatostatin (——) (0.1 µg/min) (n = 10) or saline (---) (n = 6) was given for 15 min. Means and s.e.m. are shown. Asterisks indicate probability levels of random difference. * $p < 0.05$; ** $p < 0.01$.



There was no difference in basal plasma glucose concentration between intact rats and splanchnicotomized-adrenalectomized rats, despite the enhanced basal insulin levels in the latter group. Neither somatostatin nor phentolamine induced any appreciable fluctuations in plasma glucose concentrations that could explain the changes in plasma IRI (Figs. 2 and 3).

To exclude the possibility that the failure of somatostatin to inhibit insulin secretion during α-adrenoceptor blockade in splanchnicotomized-

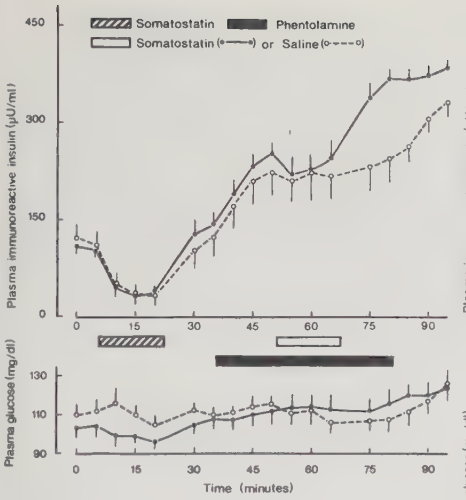


Fig 3

Arterial plasma concentrations of immunoreactive insulin and glucose in two groups of splanchnicotomized-adrenalectomized rats. Somatostatin (0.1 μg/min) was infused for 15 min in the basal state in both groups. During a subsequent administration of phentolamine (0.015 mg/min), an infusion of somatostatin (——) (0.1 μg/min) (n = 8) or saline (---) (n = 7) was given for 15 min. Means and s.e.m. are shown.

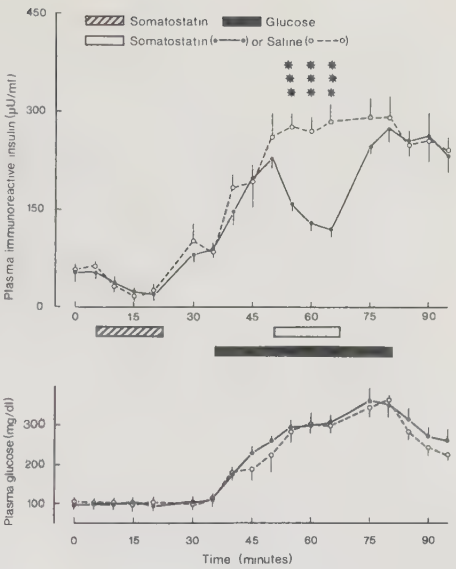


Fig. 4

Arterial plasma concentrations of immunoreactive insulin and glucose in two groups of intact rats. Somatostatin (0.1 μg/min) was infused for 15 min in the basal state in both groups. During a subsequent administration of glucose (12.5 mg/min), an infusion of somatostatin (——) (0.1 μg/min) (n = 6) or saline (---) (n = 4) was given for 15 min. Means and s.e.m. are shown. Asterisks indicate probability levels of random difference. *** $p < 0.001$.

-adrenalectomized rats was due to the high secretory rate of insulin per se, the effect of somatostatin on insulin release was studied during infusion of another secretagogue, viz glucose. Fig. 4 shows that when plasma insulin concentration in intact rats was increased by glucose to a level closely similar to that during infusion of phentolamine in splanchnicotomized-adrenalectomized rats (cf. Fig. 3), somatostatin caused a clearcut fall in plasma IRI concentration ($p < 0.001$). No difference in plasma glucose concentrations was noted between the groups in this experimental series (cf. Fig. 4).

In the next series of experiments, the dose of somatostatin infused during α-adrenoceptor blockade in splanchnicotomized-adrenalectomized rats was increased fivefold to 0.5 μg/min. Fig. 5 shows that this higher dose of somatostatin displayed the ability to clearly depress the plasma IRI concentration during the infusion of phentolamine ($p < 0.001$). Thus splanchnic denervation combined with adrenalectomy seemed to decrease but not to abolish the sensitivity of the insulin cell to somatostatin during α-adrenoceptor-blockade.

It was evident from our initial experiments that splanchnicotomized--adrenalectomized rats had higher basal insulin levels than intact rats. Since the increase in basal insulin secretion could be due to an enhanced

sensitivity to ß-adrenoceptor stimulation we tested this hypothesis by investigating the effect of ß-adrenoceptor blockade with propranolol on plasma IRI concentration in splanchnicotomized-adrenalectomized rats. Fig. 6 shows that propranolol lowered both basal and phentolamine-induced insulin secretion and that the plasma IRI concentration during α-adrenoceptor-blockade now fell significantly in response to the low dose of somatostatin ($p < 0.01$). In fact, the responses to somatostatin and phentolamine in the propranolol-treated group were closely similar to those obtained in the intact rats (cf. Fig. 2). Thus, the increased basal and phentolamine-induced insulin secretion as well as the sensitivity to somatostatin during α-adrenoceptor blockade in splanchnicotomized-adrenalectomized rats seemed to be highly dependent on intact ß-adrenoceptors.

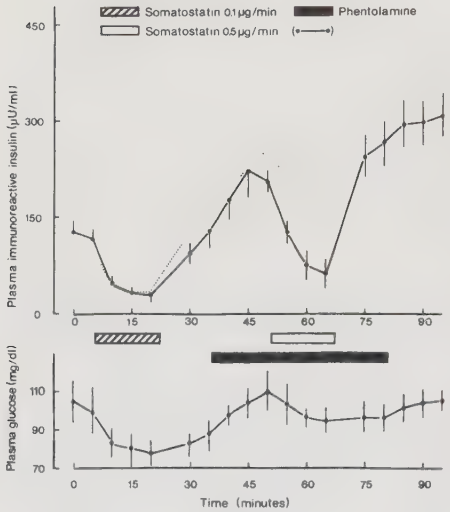


Fig. 5

Arterial plasma concentrations of immunoreactive insulin and glucose in splanchnicotomized-adrenalectomized rats. Somatostatin (0.1 µg/min) was infused for 15 min in the basal state. During a subsequent administration of phentolamine (0.015 mg/min), an infusion of somatostatin was given at a high dose (0.5 µg/min) for 15 min. Means and s.e.m. are given (n=6). The dotted line represents mean values of arterial plasma insulin concentrations in splanchnicotomized-adrenalectomized rats given the low dose of somatostatin (0.1 µg/min) during the phentolamine-infusion (depicted from Fig. 3).

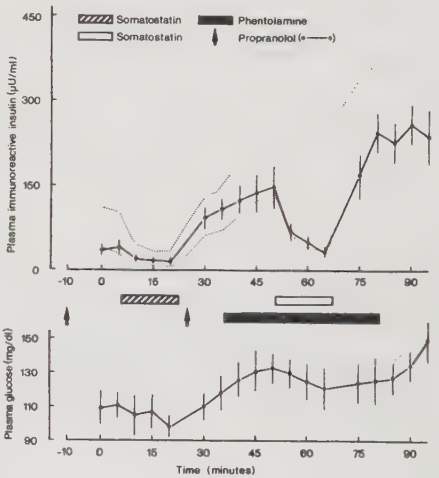


Fig. 6

Arterial plasma concentrations of immunoreactive insulin and glucose in splanchnicotomized-adrenalectomized rats subjected to ß-adrenoceptor blockade by intraperitoneal injections of propranolol (arrows). Somatostatin (0.1 µg/min) was infused for 15 min in the basal state. During a subsequent administration of phentolamine (0.015 mg/min), an infusion of somatostatin (0.1 µg/min) was given for 15 min. Means and s.e.m. are shown (n=5). The upper dotted line represents mean values of arterial plasma insulin concentrations in splanchnicotomized-adrenalectomized rats not given propranolol (depicted from Fig. 3), and the lower dotted line represents mean values of arterial plasma insulin levels in intact rats (depicted from Fig. 2). In these two experiments the infusions of somatostatin and phentolamine were identical to those in the propranolol-treated group.

The decreased sensitivity of the insulin releasing machinery to somatostatin in surgically splanchnicotomized-adrenalectomized rats during α-adrenoceptor-blockade was compared with the effects of somatostatin in rats which were chemically sympathectomized by injection of 6-hydroxydopamine and then adrenalectomized. Fig. 7 shows the changes in arterial plasma IRI and glucose concentrations from these experiments. Mean basal plasma IRI concentration was slightly higher in this group than in the control group ($p < 0.05$). Somatostatin depressed basal plasma IRI concentration by about 50% ($p < 0.01$), this inhibition being less pronounced than in intact and in splanchnicotomized-adrenalectomized rats. In contrast to in intact and in surgically splanchnicotomized-adrenalectomized rats infusion of phentolamine did not increase the plasma IRI concentrations in these 6-hydroxydopamine treated rats. During infusion of phentolamine an administration of somatostatin induced a depression of plasma IRI concentration ($p < 0.05$).

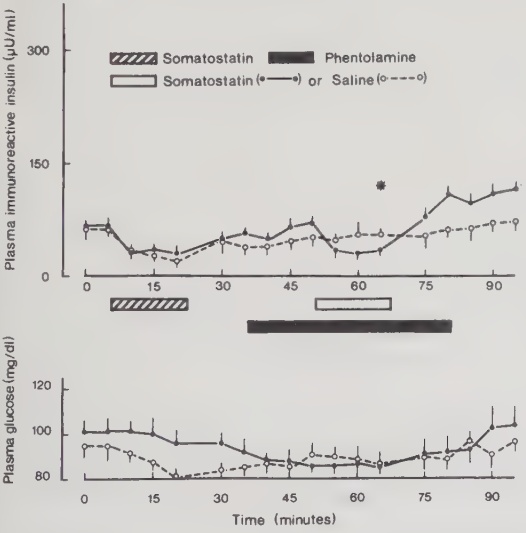


Fig. 7

Arterial plasma concentrations of immunoreactive insulin and glucose in two groups of adrenalectomized rats pretreated with 6-hydroxydopamine i.v. (65 mg/kg) two days before the experiments. Somatostatin (0.1 μg/min) was infused for 15 min in the basal state in both groups. During a subsequent administration of phentolamine (0.015 mg/min), an infusion of somatostatin (——) (0.1 μg/min) (n=5) or saline (---) (n=5) was given for 15 min. Means and s.e.m. are shown. Asterisk indicates probability level of random difference. * $p < 0.05$.

DISCUSSION

In this study the effects of somatostatin on insulin release have been investigated in anaesthetized rats subjected to different manipulations of the sympatho-adrenal system.

Insulin secretion is inhibited by stimulation of the α-adrenoceptors and augmented by stimulation of the β-adrenoceptors on the insulin cells (Porte, 1967; Lundquist, 1972a; Lundquist, 1972b; Smith & Porte, 1976; Loubatières-Mariani, Chapal, Ribes & Loubatières, 1977; Lundquist & Ericson, 1978; Gerich & Lorenzi, 1978). Blockade of the α-adrenoceptors by phentolamine *in vivo* is followed by an increased basal plasma IRI concentration in various species (cf. Lundquist, 1971), findings which are confirmed by our present observation in the rat (cf. Fig. 2). These results suggest that the α-adrenoceptors exert an inhibitory tone on the insulin cells, and that phentolamine might abolish this possible tone. In addition, phentolamine might liberate catecholamines with β-adrenoceptor-stimulating properties (Dairman & Udenfriend, 1970) which further increases insulin secretion during the α-adrenoceptor-blockade. Our finding that also splanchnicotomized-adrenalectomized rats had elevated basal plasma IRI concentrations further indicates the existence of an inhibitory tone on insulin secretion in this species, probably mediated by adrenergic nerves

and by circulating catecholamines secreted from the adrenal medulla. Despite the changes in plasma IRI, there were no differences in plasma glucose. Splanchnicotomy in monkeys (Miller, 1970) or dogs (Girardier, Seydoux, Berger & Veicsteinas, 1978) was not followed by raised plasma insulin levels which might possibly be explained by the fact that the adrenal glands were left intact in the latter experiments.

The markedly elevated basal plasma IRI concentration in splanchnicotomized-adrenalectomized rats was depressed to a level seen in intact rats by propranolol. This observation may suggest that the increased basal insulin secretion in splanchnicotomized-adrenalectomized rats is dependent on intact β-adrenoceptors. In these rats circulating amines with β-receptor--stimulating properties may thus be of great importance for basal insulin release when the inhibitory α-tone is abolished, particularly if a functional increase of the ratio of β-adrenoceptors to α-adrenoceptors has evolved (Nickerson & Kunos, 1977). This raises the question of the relative importance of α- and β-adrenoceptors in controling insulin secretion. It should be noted that blockade of the β-adrenoceptors by propranolol may negatively modulate the insulin secretory response also to other secretagogues than β-adrenergic stimulators such as glucose and sulphonylurea (Cerasi, Luft & Efendić, 1972; Sirek, Sirek & Policova, 1975). Thus, a pure β-adrenergic stimulation may not be the sole mechanism behind the enhanced plasma insulin concentration in splanchnicotomized-adrenalectomized rats. Furthermore, it cannot be excluded that splanchnicotomy may involve impairment of non-adrenergic neurons (Lundberg, Hökfelt, Nilsson, Terenius, Rehfeld, Elde & Said, 1978; Larsson, 1979) which normally might exert inhibitory influences on insulin secretion, perhaps through the adenylate cyclase--cyclic AMP system, which is supposed to be related to the β-adrenoceptors (Kuo, Hodgins & Kuo, 1973). Cutting these neurons might then lead to an increased insulin secretion through a propranolol-sensitive insulin secretory pathway.

Thus, these results suggest that normally the splanchnic nerves exert an inhibitory tone on basal insulin release in the anaesthetized rat, but the existence of non-adrenergic parts of this tone cannot be excluded. To further elucidate this inhibitory tone rats pretreated with 6-hydroxydopamine were studied.

6-Hydroxydopamine is selectively accumulated in catecholamine-containing neurons, where it displaces the natural transmitters and destroys the nerve terminals (Kostrzewa & Jacobowitz, 1974). The dose of 6-hydroxydopamine used in this study has previously been shown to give a chemical sympathectomy (Kostrzewa & Jacobowitz, 1974). Our chemically sympathectomized-adrenalectomized rats had slightly higher basal plasma IRI concentrations than the intact control rats which illustrates the inhibitory tone exerted by the sympatho-adrenal system on the insulin cell in the rat. Similar results after chemical sympathectomy were recently obtained in the conscious rat (unpublished), showing that anaesthesia *per se* did not cause this effect.

Our results are in agreement with a previous long-term study in the rat showing that basal plasma IRI concentration was enhanced 2-6 weeks after chemical sympathectomy by 6-hydroxydopamine (Burr, Jackson, Culbert, Sharp, Felts & Olson, 1974). However, the finding that the basal IRI concentration in chemically sympathectomized-adrenalectomized rats was markedly lower than that in splanchnicotomized-adrenalectomized rats emphasizes that the high IRI levels in the latter group probably cannot be explained solely by the elimination of an adrenergic inhibitory tone.

From studies *in vivo* in the dog and *in vitro* on pancreatic pieces from the rat it has been suggested that somatostatin partly exerts its effects on insulin release through stimulation of α-adrenoceptors (Smith, Woods &

Porte, 1976; Basabe, Cresto & Aparicio, 1977). This suggestion is not in accordance with results from several other reports (Efendić & Luft, 1975; Kaneto et al., 1978; Schmitt et al., 1979; Järhult, Ahrén & Lundquist, 1979; Hara, Patton & Gerich, 1979). The present investigation again showed that insulin release is inhibited by somatostatin both in the basal state and during α-adrenoceptor blockade in normal rats, thus further making it unlikely that somatostatin exerts its effects solely via stimulation of the α-adrenoceptors. Further, the increased basal insulin release in splanchnicotomized-adrenalectomized rats was readily inhibited by somatostatin. However, the sensitivity of the insulin cells to somatostatin was clearly depressed during α-adrenoceptor blockade in these rats in that the dose of somatostatin had to be elevated to detect an inhibitory effect of the peptide on insulin release. This reduced response to somatostatin was presumably not due to an increased insulin secretion per se since a pronounced glucose-induced insulin release was effectively inhibited by the same, low dose of somatostatin. However, during propranolol treatment the sensitivity to somatostatin was retained, indicating that propranolol-sensitive insulin secretory pathways show a complex interaction with somatostatin on insulin release in these rats.

In contrast to splanchnicotomized-adrenalectomized rats somatostatin exerted insulin lowering effect in the lower dose in chemically sympathectomized-adrenalectomized rats also in the presence of phentolamine. This difference may partly be explained by a phentolamine-induced liberation of catecholamines (Dairman & Udenfriend, 1970). This liberation must be greater in splanchnicotomized-adrenalectomized rats than in 6-hydroxydopamine treated rats, and the resulting insulinotropic β-adrenoceptor stimulatory effects of these amines, including noradrenaline (Lundquist, 1972b), counteract the inhibitory response to somatostatin during α-adrenoceptor blockade. This explanation seems to be further corroborated by the observation that the response to somatostatin in the lower dose during α-adrenoceptor-blockade was retained in splanchnicotomized-adrenalectomized rats subjected to β-adrenoceptor-blockade since the β-adrenoceptor-blockade inhibits the effects of these amines. It should, however, be emphasized that somatostatin in the lower dose inhibited insulin release during α--adrenoceptor-blockade in normal rats where phentolamine probably induces a release of catecholamines of comparable magnitude as in splanchnicotomized-adrenalectomized rats. Therefore, other influences such as the events induced by abolishment of the inhibitory sympathetic tone and perhaps by cutting the non-adrenergic neurons in the splanchnic nerve add to the effect of the catecholamines in splanchnicotomized-adrenalectomized rats during α-adrenoceptor-blockade. These influences may then change the integrity of the insulin cell to a state where the sensitivity to somatostatin is decreased. The exact mechanism behind this decreased sensitivity to somatostatin remains to be elucidated.

ACKNOWLEDGEMENTS

The expert technical assistance of Åsa Amilon, Peter Okmark and Monica Radnell is gratefully acknowledged. This study was supported by grants from the Swedish Medical Research Council, Nordisk Insulinfond and Maggie Stephen's Foundation.

REFERENCES

BASABE, J.C., CRESTO, J.C. & APARICIO, N. (1977). Studies on the mode of action of somatostatin on insulin secretion. Endocrinology 101, 1436-1443.

BLOOM, S.R. & EDWARDS, A.V. (1975). The release of pancreatic glucagon and inhibition of insulin in response to stimulation of the sympathetic innervation. J. Physiol. 253, 157-173.

BRUSS, M.L. & BLACK, A.L. (1978). Enzymatic microdetermination of glycogen. Anal. Biochem. 84, 309-312.

BURR, I.M., JACKSON, A., CULBERT, S., SHARP, R., FELTS, P. & OLSON, W. (1974). Glucose intolerance and impaired insulin release following 6-hydroxydopamine administration to intact rats. Endocrinology 94, 1072-1076.

CERASI, E., LUFT, R. & EFENDIĆ, S. (1972). Effect of adrenergic blocking agents on insulin response to glucose infusion in man. Acta endocr., Copenh 69, 335-346.

DAIRMAN, W. & UDENFRIEND, S. (1970). Effect of ganglionic blocking agents on the increased synthesis of catecholamines resulting from α-adrenergic blockade or exposure to cold. Biochem. Pharmac. 19, 979-984.

EFENDIĆ, S. & LUFT, R. (1975). Studies on the mechanism of somatostatin action on insulin release in man. I. Effect of blockade of α-adrenergic receptors. Acta endocr., Copenh.78, 516-523.

ESTERHUIZEN, A.C., SPRIGGS, L.B. & LEVER, M.D. (1968). Nature of islet-cell innervation in the cat pancreas. Diabetes 17, 33-36.

GALBO, H., CHRISTENSEN, N.J. & HOLST, J.J. (1977). Catecholamines and pancreatic hormones during autonomic blockade in exercising man. Acta physiol. scand. 101, 428-437.

GERICH, J.E. & LORENZI, M. (1978). The role of the autonomic nervous system and somatostatin in the control of insulin and glucagon secretion. Frontiers in Neuroendocrinol. 5, 265-288.

GIRARDIER, L., SEYDOUX, J., BERGER, M. & VEICSTEINAS, A. (1978). Selective pancreatic nerve section. An investigation of neural control of glucagon release in the conscious unrestrained dog. J. Physiol., Paris 74, 731-735.

HARA, M., PATTON, G.S. & GERICH, J.E. (1979). Studies on the mechanism of action of somatostatin on the endocrine pancreas. I. Interaction with adrenergic receptors. Endocrinology 105, 801-805.

HEDING, L. (1966). A simplified insulin radioimmunoassay method. In Labelled proteins in tracer studies, ed. DONATO, L., MILHAUD, G. & SIRCHIS, J. pp. 345-350. Brussels: Euratom.

HÖKFELT, T., EFENDIĆ, S., HELLERSTRÖM, C., JOHANSSON, O., LUFT, R. & ARIMURA, A. (1975). Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special references to the A_1-cells of the pancreatic islets and to the hypothalamus. Acta endocr., Copenh. 80. Suppl. 200.

JÄRHULT, J., AHREN, B. & LUNDQUIST, I. (1979). Inhibitory effect of somatostatin on insulin secretion during α-adrenergic blockade in three different species. Acta endocr., Copenh. 92, 166-173.

JÄRHULT, J. & HOLST, J.J. (1978). Reflex adrenergic control of endocrine pancreas evoked by unloading of carotid baroreceptors in cats. Acta physiol. scand. 104, 188-202.

JÄRHULT, J. & HOLST, J. (1979). The role of the adrenergic inhibition to the pancreatic islets in the control of insulin release during exercise in man. Pflügers Arch. ges. Physiol. 383, 41-45.

KANETO, A., KAJINUMA, H., KANEKO, T. & KOSAKA, K. (1978). Effect of phentolamine on the somatostatin-induced inhibition of glucagon and insulin secretion. Metabolism 27, 901-910.

KOSTRZEWA, R.M. & JACOBOWITZ, D.M. (1974). Pharmacological actions of 6-hydroxydopamine. Pharmac. Rev. 26, 199-288.

KUO, W.N., HODGINS, D.S. & KUO, J.F. (1973). Adenylate cyclase in islets of Langerhans. Isolation of islets and regulation of adenylate cyclase activity by various hormones and agents. J. biol. Chem. 248, 2705-2711.

LARSSON, L.-I. (1979). Innervation of the pancreas by substance P, enkephalin, vasoactive intestinal polypeptide and gastrin/CCK immunoreactive neurons. J. Histochem. Cytochem. 27, 1283-1285.

LOUBATIÈRES-MARIANI, M.M., CHAPAL, J., RIBES, G. & LOUBATIÈRES, A.-L. (1977). Discrepancies in the response of the insulin secreting cells of the dog and rat to different adrenergic stimulating agents. Acta diabetol. lat. 14, 144--155.

LUFT. R., EFENDIĆ, S. & HÖKFELT, T. (1978). Somatostatin - both hormone and neurotransmitter? Diabetologia 14, 1-13.

LUNDBERG, J.M., HÖKFELT, T., NILSSON, G., TERENIUS, L., REHFELD, J., ELDE, R. & SAID, S. (1978). Peptide neurons in the vagus, splanchnic and sciatic nerves. Acta physiol. scand. 104, 499-501.

LUNDQUIST, I. (1971). Insulin secretion. Its regulation by monoamines and acid amyloglucosidase. Acta physiol. scand. suppl. 372, 1-47.

LUNDQUIST, I. (1972a). Interaction of amines and aminergic blocking agents with blood glucose regulation. I. β-Adrenergic blockade. Europ. J. Pharmacol. 18. 213-224.

LUNDQUIST, I. (1972b). Interaction of amines and aminergic blocking agents with blood glucose regulation. II. α-Adrenergic blockade. Europ. J. Pharmacol. 18, 225-235.

LUNDQUIST, I. & ERICSON, L.E. (1978). β-Adrenergic insulin release and adrenergic innervation of mouse pancreatic islets. Cell Tiss. Res. 193, 73-85.

MILLER, R.E. (1970). Effects of vagotomy or splanchnicotomy on blood insulin and sugar concentrations in the conscious monkey. Endocrinology 86, 642-651.

MILLER, R.E. (1975). Neural inhibition of insulin secretion from the isolated canine pancreas. Am. J. Physiol. 229, 144-149.

NICKERSON, M. & KUNOS, G. (1977). Discussion of evidence regarding induced changes in adrenoceptors. Fedn. Proc. 36, 2580-2583.

PORTE Jr, D. (1967). A receptor mechanism for the inhibition of insulin release by epinephrine in man. J. clin. Invest. 46, 86-94.

PORTE, Jr, D., GIRARDIER, L., SEYDOUX, J., KANAZAWA, U. & POSTERNAK, J. (1973). Neural regulation of insulin secretion in the dog. J. clin. Invest. 52, 210-214.

SCHMITT, J.K., LORENZI, M., GERICH, J.E., BOHANNON, N.V., KARAM, J.H. & FORSHAM, P.H. (1979). Phentolamine and the action of somatostatin in man. J. clin. Endocrin. Metab. 48, 880-882.

SIREK, O.V., SIREK, A. & POLICOVA, Z. (1975). Inhibition of sulphonylurea--stimulated insulin secretion by beta adrenergic blockade. Diabetologia 11, 269-272.

SMITH, P.H. & PORTE Jr, D. (1976). Neuropharmacology of the pancreatic islets. Ann. Rev. Pharmacol. Toxicol. 16, 269-285.

SMITH, P.H., WOODS, S.C. & PORTE Jr, D. (1976). Phentolamine blocks the somatostatin-mediated inhibition of insulin secretion. Endocrinology 98, 1073-1076.

Insulin Secretion Induced by Glucose and by Stimulation of β_2-Adrenoceptors in the Rat
Different Sensitivity to Somatostatin

Bo Ahrén, Johannes Järhult and Ingmar Lundquist

Departments of Pharmacology and Surgery, University of Lund, Sweden

SUMMARY

The effects of somatostatin on insulin secretion stimulated by glucose or by the selective β_2-adrenoceptor agonist terbutaline were studied *in vivo* in the anaesthetized rat. Infusion of low doses of glucose (5 mg/min) or terbutaline (2 µg/min) caused slight stimulation of insulin secretion, whereas infusion of higher doses of glucose (12.5 mg/min) or terbutaline (200 µg/min) yielded higher rates of insulin release. In both instances plasma insulin concentrations were of comparable magnitudes immediately prior to somatostatin infusion.

Somatostatin (0.1 µg/min) inhibited the insulin response to glucose and terbutaline, both at the low and high secretory rates. However, the inhibitory effect of somatostatin was much more pronounced on insulin release during glucose infusion than during infusion of terbutaline. Thus, at the high rate of insulin secretion somatostatin depressed plasma insulin by 46% during glucose and by 22% during terbutaline infusion. The results at the low rate of insulin secretion were 66% and 48%, respectively. Both at high and low secretory rates somatostatin depressed plasma insulin levels more pontently during glucose infusion than during terbutaline infusion ($p < 0.001$ and $p < 0.05$, respectively). Furthermore, the plasma insulin levels during inhibition by somatostatin following terbutaline stimulation stabilized after approximately 10 min of somatostatin infusion, whereas following glucose stimulation the insulin levels continued to decline. The results suggest that somatostatin inhibits insulin secretion via mechanisms that are more closely related to the insulin secretory pathway induced by glucose than to that induced by β-adrenoceptor agonists.

INTRODUCTION

Insulin release induced by various stimuli such as glucose, arginine, sulphonylurea, isoproterenol, glucagon, carbachol, phentolamine and phosphodiesterase inhibitors is inhibited by somatostatin in a variety of species (Gerich et al. 1975, Efendić et al. 1976, Lundquist et al. 1979, Järhult

et al. 1979, Taborsky et al. 1979). Since these secretagogues probably induce secretion of insulin through mechanisms which are at least partially different from each other, it has been suggested that somatostatin exerts its inhibitory effect by influencing a late step in the insulin discharge process, e.g. the handling of Ca^{++} (Curry & Bennett 1974, Taminato et al. 1975, Fujimoto & Ensinck 1976, Oliver 1976, Grodsky et al. 1977). However, the exact mechanisms responsible for the effect of somatostatin on insulin release have not yet been established. In order to further characterize the effect of somatostatin on insulin release we have investigated *in vivo* in the rat the influence of the peptide on insulin secretion stimulated by two different insulin secretagogues, glucose and the selective β_2-adrenoceptor agonist terbutaline (Bergman et al. 1969). It was found that the insulin secretion induced by glucose was more sensitive to somatostatin inhibition than that induced by terbutaline.

METHODS

Animals. The experiments were carried out on adult male Wistar rats (270--295 g b.w.) purchased from Møllegaard Avlslaboratorium, Skensved, Denmark. They were kept on standard pellets (Astra-Ewos, Södertälje, Sweden) and tap water *ad libitum*.

Drugs. Synthetic cyclic somatostatin (Beckman Ltd., Geneva, Switzerland) was dissolved in saline with the addition of 0.1% gelatine to avoid adsorbtion. Terbutaline sulfate (2-*tert*-butylamino-(3,5-dihydroxyphenylethanol)sulfate) was a gift from Draco AB, Lund, Sweden. D-glucose was obtained from British Drug Houses Ltd., Poole, England and nembutalsodium (sodium pentobarbitone) from ACO AB, Solna, Sweden.

Experiments. The rats were anaesthetized i.p. with pentobarbitone (60 mg/kg b.w.). Polyethylene catheters were inserted into the right femoral vessels. The venous catheter was connected to a pump and used for infusions of drugs and the arterial catheter was used for collection of blood samples. After two control blood samples, a 50 min infusion of glucose or terbutaline was started. Twentyfive min after the start of this infusion, somatostatin (0.1 µg/min) or saline was given as a continuous i.v. infusion for 15 min. All infusions were administered at a rate of 0.1 ml/min. The periods for the different infusions and the intervals between the blood samples are easily seen in the figures.

Determination of insulin and glucose. Plasma immunoreactive insulin concentrations (IRI) were measured with radioimmunoassay (Heding 1966). Plasma glucose concentrations were determined with a glucose oxidase method (Bruss & Black 1978).

Statistics. All data are presented as mean values $\pm$ s.e.m. Student's *t*-test and *t*-test for paired observations were employed for the statistical evaluation. For each experimental series the change in insulin secretion during somatostatin or saline infusion was calculated as the difference between the insulin concentration immediately prior to the somatostatin infusion and that at the end of the infusion. The effects of somatostatin were calculated as the difference between these obtained values. To normalize the two groups in each series, the values were expressed as percent of pre-infusion concentrations.

RESULTS

In the first series of experiments glucose was infused at the low dose

of 5 mg/min (Fig. 1). Insulin secretion was immediately stimulated by glucose and plasma IRI levels reached a peak value approximately 15 min after the start of the infusion. Thereafter, plasma insulin levels gradually declined, despite the continuous infusion of glucose. Upon infusion of somatostatin (0.1 µg/min), plasma insulin levels decreased promptly, and 15 min after the start of the somatostatin infusion it averaged only 6 ± 2 µU/ml; the value immediately prior to somatostatin being 92 ± 22 µU/ml ($p < 0.001$). During infusion of saline, mean plasma insulin levels decreased by 27%, and it can be calculated that in response to somatostatin insulin secretion was diminished by 66 ± 7 % ($p < 0.001$). An immediate off--response in insulin secretion was seen when the somatostatin infusion was stopped. After the glucose infusion, plasma insulin levels were lowered promptly.

Plasma glucose increased about 2 mmol/l in response to glucose. No appreciable changes in plasma glucose were observed during the infusion of somatostatin despite the fact that plasma insulin changed markedly.

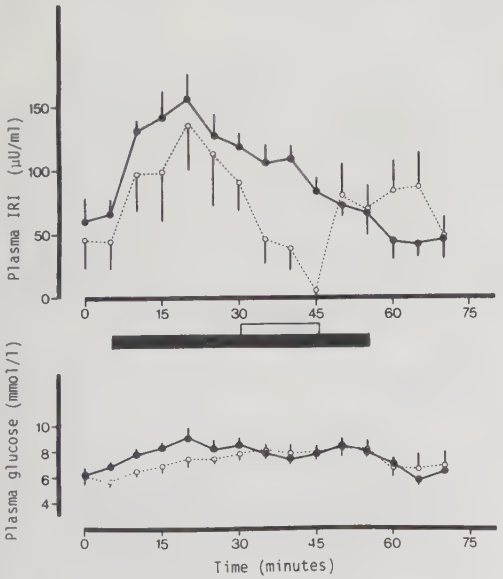


Fig. 1

Changes in arterial plasma concentrations of immunoreactive insulin and glucose in two groups of rats. Glucose, 5 mg/min, was infused for 50 min in both groups and during this infusion either somatostatin (---), 0.1 µg/min, or saline (——) was given for 15 min. There were 5 animals in each group. Means and standard error of the means are shown.

Fig. 2 shows changes in plasma insulin and glucose concentrations in response to infusion of terbutaline at the low dose of 2 µg/min. Plasma IRI concentrations increased slightly ($p < 0.05$) in response to terbutaline and reached a plateau after about 25 min of infusion. Infusion of somatostatin (0.1 µg/min) immediately decreased plasma IRI; the IRI levels being depressed from 70 ± 12 µU/ml to 25 ± 10 µU/ml ($p < 0.001$). This decremental effect seemed to be stabilized after 10 min of somatostatin administration. During the infusion of somatostatin plasma insulin decreased by 65% and and during infusion of saline it fell by 17%. Therefore, the true decrease in plasma insulin in response to somatostatin was 48 ± 5% ($p < 0.001$). This effect of somatostatin was weaker ($p < 0.05$) than that during infusion of glucose (5 mg/min). After terminating the somatostatin infusion, plasma IRI returned to pre-infusion values.

There was a continuous slow increase in plasma glucose concentrations during the course of these experiments. No fluctuations of plasma glucose was seen in response to somatostatin.

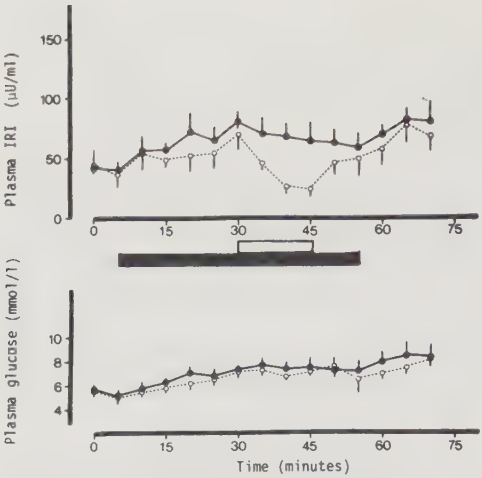


Fig. 2

Changes in arterial plasma concentrations of immunoreactive insulin and glucose in two groups of rats. Terbutaline, 2 μg/min, was infused for 50 min in both groups, and during this infusion either somatostatin (---), 0.1 μg/min, or saline (——) was given for 15 min. There were 5 animals in each group. Means and standard error of the means are shown.

In the following series of experiments, a higher rate of insulin secretion was induced by increasing the doses of terbutaline and glucose. Fig. 3 shows the changes in plasma IRI and glucose concentrations in response to infusion of glucose at the dose of 12.5 mg/min. This glucose infusion raised plasma glucose concentrations by approximately 6-7 mmol/l and plasma IRI levels were elevated to 239 $\pm$ 13 μU/ml ($p < 0.001$) immediately prior to somatostatin infusion. Somatostatin (0.1 μg/min) markedly inhibited this large insulin secretion; plasma insulin concentrations were 111 $\pm$ 7 μU/ml ($p < 0.001$) after 15 minutes of infusion. In response to somatostatin, plasma IRI concentrations decreased by 46 $\pm$ 2% ($p < 0.001$). Immediately after stopping the somatostatin infusion, plasma insulin returned to the preinfusion level.

There were no apparent changes in plasma glucose in response to the infusion of somatostatin.

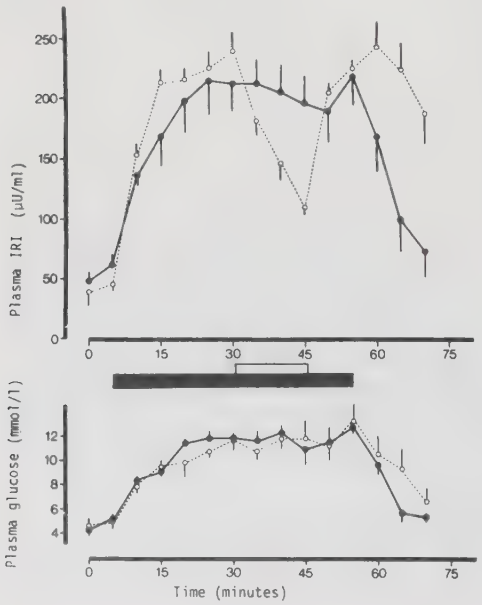


Fig. 3

Changes in arterial plasma concentrations of immunoreactive insulin and glucose in two groups of rats. Glucose, 12.5 mg/min, was infused for 50 min in both groups, and during this infusion either somatostatin (---), 0.1 μg/min, or saline (——) was given for 15 min. There were 5 animals in each group. Means and standard error of the means are shown.

Fig. 4 shows the changes in plasma IRI and glucose concentrations in response to a high dose of terbutaline (200 µg/min). This large dose of terbutaline was necessary to obtain an increase in insulin secretion similar to that after the high glucose infusion. Plasma insulin concentrations rose markedly in response to this dose of terbutaline, and this hyperinsulinemia was depressed by somatostatin (0.1 µg/min). Terbutaline increased plasma IRI levels to 167 ± 15 µU/ml ($p < 0.001$) seen immediately prior to somatostatin infusion. Somatostatin depressed this value to 118 ± 19 µU/ml ($p<0.001$). However, the influence of somatostatin on insulin secretion induced by terbutaline was less pronounced than on that induced by glucose (cf. Fig. 3). Thus, the plasma IRI concentrations decreased by 22 ± 2% during somatostatin infusion, which is lower than that during infusion of the high dose of glucose ($p < 0.001$). It was also noted that the depression of insulin secretion induced by somatostatin was stabilized after 10 min.

There were no apparent changes in plasma glucose concentrations in response to terbutaline or somatostatin.

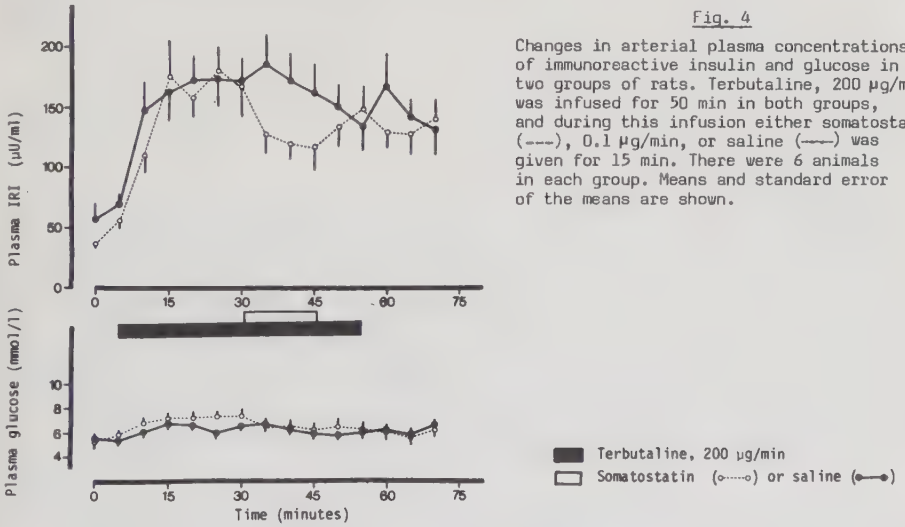


Fig. 4

Changes in arterial plasma concentrations of immunoreactive insulin and glucose in two groups of rats. Terbutaline, 200 µg/min, was infused for 50 min in both groups, and during this infusion either somatostatin (---), 0.1 µg/min, or saline (——) was given for 15 min. There were 6 animals in each group. Means and standard error of the means are shown.

DISCUSSION

Insulin secretion in different mammalian species including man is stimulated by ß-adrenoceptor agonists, e.g. isoproterenol, salbutamol or orciproterenol (Loubatières et al. 1971, Raptis et al. 1977, Taborsky et al. 1979). The mechanism behind the insulin releasing effect of ß-adrenoceptor agonists is probably different from that of glucose and has tentatively been explained by formation of cAMP (Kuo et al. 1973), leading to a stimulated translocation of Ca^{++} and a subsequent increase in insulin release (Brisson et al. 1973, Siegel et al. 1980). On the other hand, glucose is considered to induce release of insulin by direct stimulation of Ca^{++}-uptake, or by inhibition of Ca^{++}-efflux and/or translocation of Ca^{++} from intracellular storage sites (Hellman et al. 1979), although mediation by cAMP has also been suggested (Efendić et al. 1975).

The present study shows that the selective $ß_2$-adrenoceptor agonist terbutaline stimulates insulin secretion in the rat. At a lower dose, terbutaline induced a gradual increase in plasma IRI levels whereas at a high

dose, plasma IRI levels increased promptly in response to the drug. Plasma glucose concentrations were not subjected to any impressive changes in response to terbutaline. However, taking the elevated plasma insulin levels into account, a relative hyperglycemia might exist during terbutaline infusion. Thus, this drug might influence insulin release also indirectly, e.g. by a ß-adrenoceptor mediated delivery of glucose from the liver. Tentatively, somatostatin might only inhibit this glucose-induced part of insulin secretion during terbutaline administration.

It has been shown previously that somatostatin can depress insulin secretion induced by ß-adrenoceptor agonists in man (Raptis et al. 1977) and dogs (Taborsky et al. 1979) and this study shows that insulin secretion in response to ß-adrenoceptor stimulation in the rat is depressed by somatostatin. However, the capability of somatostatin to reduce insulin secretion during terbutaline is lower than its capability to reduce the insulin response to glucose. This was shown at high and low secretory rates. Furthermore, insulin secretion pattern following inhibition by somatostatin was different during glucose infusion than during terbutaline infusion.

Our data in the rat seem compatible with those recently reported in the dog (Taborsky et al. 1979). These authors showed that a dose of somatostatin which completely eliminated the insulin response to glucose did not abolish the effect of isoproterenol on insulin release. However, the insulin secretory response to glucose was less than that caused by isoproterenol and the difference in the inhibition may therefore be due to difference in the magnitude of stimulation.

The mode of action of somatostatin on insulin secretion cannot be deduced from the present study. The results suggest, however, that somatostatin inhibits the effects of various insulin secretagogues differently at least from a quantitative point of view. During terbutaline infusion, somatostatin depressed plasma insulin by about 50 µU/ml, whereas during glucose infusion, it suppressed plasma insulin levels to a much larger extent. It is of interest to note that 50 µU/ml is the plasma insulin level at basal conditions in these rats. It might therefore be speculated, that the effect of somatostatin during terbutaline infusion represents the inhibition by somatostatin on basal insulin secretion. We have thus shown previously that basal insulin levels in the rat are very potently depressed by the same dose of somatostatin as used in the present study (Järhult et al. 1979).

In conclusion, our findings indicate that the effect on insulin secretion by somatostatin is more linked to the effects of glucose than to those of terbutaline. It has been demonstrated recently that insulin secretion in rats subjected to surgical splanchnicotomy and adrenalectomy during α-adrenoceptor blockade is less sensitive to somatostatin than insulin secretion in intact rats (Ahrén et al. 1981), and, further, that adrenergically mediated glucagon secretion in cats and calves is difficult to suppress with somatostatin (Järhult et al. 1978, Bloom & Edwards 1978). Whether the observation that hormone secretion in response to adrenergic stimulation is comparably insensitive to somatostatin represents a general phenomenon remains to be further elucidated.

ACKNOWLEDGEMENTS

The authors are very grateful to Mrs. Monica Radnell and Mr. Peter Okmark for their skilled technical assistance. This study was supported by grants from Nordisk Insulinfond and from the Swedish Medical Research Council (14P-4289, 14X-4286, and 04X-2210).

REFERENCES

AHRÉN, B., JÄRHULT, J. & LUNDQUIST, I. 1981. Influence of the sympatho-adrenal system and somatostatin on the secretion of insulin in the rat. J Physiol in press

BERGMAN, J., PERSSON, H. & WETTERLIN, K. 1969. Two new groups of selective stimulants of adrenergic β-receptors. Experientia 25:899-890.

BLOOM S.R. & EDWARDS, A.V. 1978. Certain pharmacological characteristics of the release of pancreatic glucagon in response to stimulation of the splanchnic nerves. J Physiol 280:25-35.

BRISSON, G.R., MALAISSE-LAGAE, F. & MALAISSE, W. 1972. The stimulus-secretion coupling of glucose-induced insulin release. VII. A proposed site of action for adenosine-3'5'-cyclic monophosphate. J Clin Invest 51:232--241.

BRUSS, M.L. & BLACK, A.L. 1978. Enzymatic microdetermination of glycogen. Analyt Biochem 84:309-312.

CURRY, D.L. & BENNETT, L.L. 1974. Reversal of somatostatin inhibition of insulin secretion by calcium. Biochem Biophys Res Commun 60:1015-1019.

EFENDIC, S., GRILL, V. & LUFT, R. 1975. Inhibition by somatostatin of glucose induced 3':5'-monophosphate (cyclic AMP) accumulation and insulin release in isolated pancreatic islets of the rat. FEBS Letters 55:131-133.

EFENDIC, S., LUFT, R. & CLARO, A. 1976. Studies on the mechanism of somatostatin action on insulin release in man. II. Comparison of the effects of somatostatin on insulin release induced by glucose, glucagon and tolbutamide. Acta Endocrinol (Kbh) 81:743-752.

FUJIMOTO, W.Y. & ENSINCK, J. 1976. Somatostatin inhibition of insulin and glucagon secretion in rat islet culture. Reversal by ionophore A23187. Endocrinology 98:259-262.

GERICH, J.E., LOVINGER, R. & GRODSKY, G.M. 1975. Inhibition by somatostatin of glucagon and insulin release from the perfused rat pancreas in response to arginine, isoproterenol and theophylline: Evidence for a preferential effect on glucagon secretion. Endocrinology 96:749-754.

GRODSKY, G.M., LUNDQUIST, I., FANSKA, R. & PICTET, R. 1977. Interrelationship of calcium and somatostatin in the secretion of insulin and glucagon. In: FOA, P.P., BAJAJ, S.S. & FOA, N.L. (eds). Glucagon, its role in physiology and clinical medicine. Springer Verlag, New York, Heidelberg, Berlin, pp. 215-230.

HEDING, L. 1966. A simplified insulin radioimmunoassay method. In: DONATO, L., MILHAUD, G. & SIRCHIS, J. (eds). Labelled proteins in tracer studies. Euratom, Brussels, pp. 345-350.

HELLMAN, B., ANDERSSON, T., BERGGREN, P.O., FLATT, P., GYLFE, E. & KOHNERT, K.D. 1979. The role of calcium in insulin secretion. In: DUMONT, J. & NUNEZ, J. (eds). Hormones and cell regulation, vol III. Elsevier/ North Holland Biomedical Press, Amsterdam, pp. 69-96.

JÄRHULT, J., AHRÉN, B., ERICSSON, M. & HOLST, J. 1978. Failure of somatostatin to eliminate the glucagon release induced by baroreceptor unloading in the cat. Acta Physiol Scand 103:478-480.

JÄRHULT, J., AHRÉN, B. & LUNDQUIST, I. 1979. Inhibitory effect of somatostatin on insulin secretion during α-adrenergic blockade in three different species. Acta Endocrinol (Kbh) 92:166-173.

KUO, W.N., HODGINS, D.S. & KUO, J.F. 1973. Adenylate cyclase in islets of Langerhans: Isolation of islets and regulation of adenylate cylase activity by various hormones and drugs. J Biol Chem 248:2705-2711.

LOUBATIÈRES, A., MARIANI, M.M., SOREL, G. & SAVI, L. 1971. The action of β--adrenergic blocking and stimulating agents on insulin secretion: Characterization of the type of β-receptor. Diabetologia 7:127-132.

LUNDQUIST, I., SUNDLER, F., AHRÉN, B., ALUMETS, J. & HÅKANSON, R. 1979. Somatostatin, pancreatic polypeptide, substance P, and neurotensin: Cellular distribution and effects on stimulated insulin secretion in the mouse. Endocrinology 104:832-838.

OLIVER, J.R. 1976. Inhibition of calcium uptake by somatostatin in isolated rat islets of Langerhans. Endocrinology 99:910-913.

RAPTIS, S., ESCOBAR-JIMENEZ, R., ROSENTHAL, J., DITSCHUNEIT, H.H. & PFEIFFER, E.F. 1977. Somatostatin modulation of pancreatic glucagon, insulin, glucose and free fatty acids following beta-adrenergic stimulation. J Clin Endocrinol Metab 44:1088-1093.

SIEGEL, E.G., WOLLHEIM, C.B., KIKUCHI, M., RENOLD, A. & SHARP, G.W.G. 1980. Dependency of cyclic AMP-induced insulin release on intra- and extracellular calcium in rat islets of Langerhans. J Clin Invest 65:233-241.

TABORSKY, G.J., SMITH, P.H., HALTER, J.B. & PORTE, D. 1979. Glucose infusion potentiates the acute insulin response to nonglucose stimuli during the infusion of somatostatin. Endocrinology 105:1215-1220.

TAMINATO, T., SEINO, Y., GOTO, Y. & IMURA, H. 1975. Interaction of somatostatin and calcium in regulating insulin release from isolated pancreatic islets of rats. Biochem Biophys Res Commun 66:928-934.

THE ROLE OF INTRACELLULAR AMINES IN THE REGULATION OF ISLET CELL FUNCTION

Ingmar Lundquist, Bo Ahrén, Rolf Håkanson and Frank Sundler

Departments of Pharmacology and Histology, University of Lund, Lund, Sweden

The role of islet cell arylethylamines such as dopamine and 5-hydroxytryptamine (5-HT) in the regulation of the storage and secretion of insulin and glucagon, has been intensively investigated (cf 16,18,21,32,33,35) ever since these amines were first identified by their formaldehyde-induced fluorescence in pancreatic islets from different mammals such as guinea-pig, cat, dog, and horse (6). It should be noted, however, that the insulin and glucagon cells of common laboratory animals as e.g. the adult mouse, rat, rabbit and hamster are devoid of formaldehyde-induced fluorescence (3). This, however, does not exclude that very low, or histochemically undetectable concentrations of dopamine and/or 5-HT may exist in their islets (11,36). Further, the insulin and glucagon cells of these species are known to have the ability to take up and decarboxylate exogenous L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan (L-5-HTP) to dopamine and 5-HT, respectively, and to store the amines intracellularly (3,4). Available evidence indicate that the amine, whether formed from endogenous or exogenous precursors, is stored together with the hormone in the secretory granules of the insulin and glucagon cells (4,5,10,17). The functional role of the granule-located amine is still far from understood. Judged from experiments in the guinea-pig, whose insulin cells contain large amounts of 5-HT, the amine does not seem to be important for insulin storage, since manipulation of the amine concentration did not affect the insulin concentration and *vice versa* (26,28). Nevertheless a large number of studies in different species under a variety of experimental conditions suggests a role for the amine in the secretory machinery of the insulin cells (cf 16,18,21,32,33,35). Most of the results obtained speak in favour of an inhibitory mode of action of the amine on insulin release. However, because of species differences and differences in experimental conditions (allowing mixed intra- and extracellular actions of the amines on insulin secretion), these data being indirect and equivocal, must be interpreted with caution. In fact, the search for a function of the intracellular amines is still in an initial stage.

The present investigation deals with four different aspects on the role of intracellular amines in the regulation of islet cell function. The first concerns the nervous regulation of the 5-HT stores of the guinea-pig insulin cells. The second concerns the accumulation of dopamine or 5-HT in mouse islet cells following the *in vivo* administration of L-DOPA, L-5-HTP, dopamine, or 5-HT. The third concerns the effect of L-DOPA-induced dopamine accumulation in mouse islet cells on the stimulated release of insulin and glucagon. The fourth, finally, concerns the possibility that amines in insulin cells interfere with calcium translocation processes.

MATERIALS AND METHODS

L-DOPA, dopamine, L-5-HTP and 5-HT were obtained from Fluka AG, Buchs, Switzerland; Pargyline, a monoamine oxidase inhibitor, from Abbot Lab., North Chicago, Ill. USA; Benserazide (Ro-4-4602), an inhibitor of the aromatic amino acid decarboxylase, from Hoffmann-La Roche Ltd., Basel, Switzerland; and L-isopropylnoradrenaline (L-IPNA) and 6-hydroxydopamine (6-OH-DA) from Hässle AB, Göteborg, Sweden. L-3-^{14}C-DOPA (6,7 mCi/mmol), 2-^{14}C-dopamine (60 mCi/mmol), and $^{45}CaCl_2$ (16,5 mCi/mg Ca) were purchased from Radiochemical Centre, Amersham, England. All other drugs and chemicals were obtained from British Drug Houses Ltd., Poole, England.

Female NMRI mice (25-35 g), female Wistar rats (250-350 g) and pigmented guinea-pigs of both sexes (350-450 g) were used. All animals were allowed free access to food and water before and throughout all experiments. Surgical procedures: Small biopsies were taken from both the lienal and the duodenal part of the pancreas of pentobarbital anaesthetized guinea-pigs. The animals were then subjected to subdiaphragmatic bilateral vagotomy (4 animals), or sham-operation (2 animals), or injected intraperitoneally with 1.35 mmol/kg of 6-OH-DA (chemical sympathectomy) 10 min after closure of the operation wound (2 animals). Final pancreatic biopsies were taken 48 h later. Blood sampling in the unanaesthetized mouse by the orbital bleeding technique and plasma glucose measurements (glucose oxidase) have been described previously (30). Immunoreactive insulin

determinations were performed by the method of Heding (12). Concentration of glucagon in small samples (25 µl) of unextracted plasma was determined by the radioimmunoassay procedure of Heding (13). The methodological procedures for fluorescence microscopy ad modum Falck and Hillarp, microspectrofluorometry, and immunohistochemistry of pancreatic hormones, have been described in previous publications (27,28). The technique for ^{45}Ca-labeling, and perifusion of isolated rat islets was performed essentially as described recently (7). The bicarbonate buffer (7) contained 10.0 mM HEPES 2,8 mM glucose 2.0 mM Ca^{2+} and 0.2% human serum albumin. Ascorbic acid in equimolar concentration to L-DOPA and benserazide, respectively, (and in control medium) was used to prevent oxidation of these drugs when they were added to the perifusion medium. About 100 islets were used in each perifusion chamber.

RESULTS

Nervous Regulation of the 5-HT Store in Guinea-Pig Insulin Cells. In the guinea-pig pancreas, yellow formaldehyde-induced fluorescence typical of 5-HT was observed in the insulin cells of the pancreatic islets (Fig. 1a). Two days after bilateral vagotomy the fluorescence intensity was greatly reduced (Fig. 1 b), whereas chemical sympathectomy brought about an increase in the fluorescence intensity (Fig. 1c). No effect was observed in sham-operated animals. Microfluorometric measurement of relative fluorescence intensities revealed that the fluorescence in the insulin cells was reduced about 50% after vagotomy and increased about 40% after chemical sympathectomy (Fig. 2).

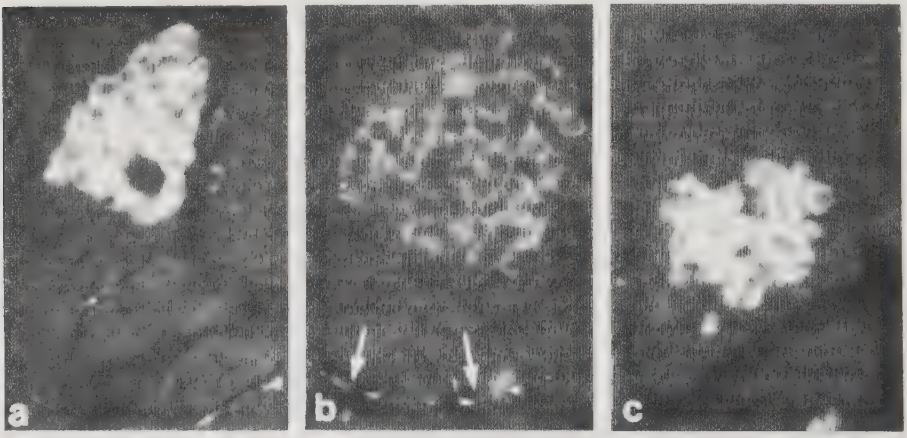


Figure 1. Formaldehyde-induced 5-HT fluorescence in guinea-pig insulin cells a) Control b) Vagotomy; reduced fluorescence c) Chemical sympathectomy; slightly increased fluorescence. Arrows indicate adrenergic nerves (X 250). See also Fig. 2.

Formation and Storage of 5-HT or Dopamine in Mouse Islet Cells. 14 mice were injected intraperitoneally with pargyline (monoamine oxidase inhibitor) 0.26 mmol/kg. Two hours later groups of 2 or 3 were given an intravenous injection of equimolar doses (0.26 mmol/kg) of L-DOPA, dopamine, L-5-HTP and 5-HT, respectively. Pancreatic biopsies were taken 2 h later. In addition 2 groups of the pargyline treated mice received benserazide (decarboxylase inhibitor) (0.85 mmol/kg) 30 min prior to amine precursor. From Fig. 3 (a and e) it appears that both L-DOPA and L-5-HTP were taken up, decarboxylated and stored in the cytoplasm of most islet cells. The precursor amino acids themselves were not stored (Fig. 3 b and e), and dopamine and 5-HT were not taken up (Fig. 3 c and f). Further it was obvious that a small population of peripheral islet cells did not accumulate 5-HT after L-5-HTP (Fig. 4 a, arrowheads). Similar results were obtained with L-DOPA (not shown). The cells that did not take up and decarboxylate amine precursors were identified as somatostatin cells by immunohistochemistry (Fig. 4 b). In another experiment groups of 4 mice were pretreated with pargyline as above. Two hours later they were injected intravenously with either L-^{14}C-DOPA or ^{14}C-dopamine (5 µCi/mouse). The mice were sacrificed after 10 or 120 min and 200-300 pancreatic islets were isolated, pooled and homogenized in 0.5 ml Hanks solution. Aliquots were counted in a liquid scintillation spectrometer. Table 1 shows that the uptake of dopamine is negligible compared to that of L-DOPA both at 10 and 120 min. The radiochemical analysis thus conformed with the fluorescence microscopy (Fig. 3).

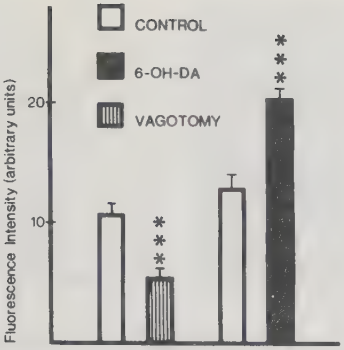


Figure 2. Relative intensity of formaldehyde-induced 5-HT fluorescence in guinea-pig insulin cells prior to and 48 h after vagotomy or chemical sympathectomy (6-OH-DA). Means ± SEM of 28-56 microspectrofluorometric readings taken from 6-8 islets of each animal. All readings were made in one session. Asterisks (***) denote $p < 0.001$.

Stimulated Insulin and Glucagon Release In Vivo after L-DOPA-Induced Dopamine Accumulation in Mouse Islet Cells. This series of experiments was designed to elucidate the effect of L-DOPA-induced dopamine accumulation in mouse islet cells on the stimulated release of insulin and glucagon. Mice were pretreated with pargyline + L-DOPA and insulin and glucagon release was stimulated by 3 different secretagogues, the β-adrenergic agonist L-IPNA, the cholinergic agonist carbachol, and the amino acid L-arginine, respectively. The control group of mice (open columns) received saline as pretreatment. A second group (striped columns) received an intraperitoneal injection of pargyline 0.26 mmol/kg 4 h, and an intravenous injection of L-DOPA (0.26 mmol/kg) 2 h prior to the experiment. The third group of mice (black columns) received a further pretreatment with pargyline (0.52 mmol/kg) 24 h before the experiment and then pargyline and L-DOPA as above. This third group was included in the experiment because ancillary experiments had shown that the

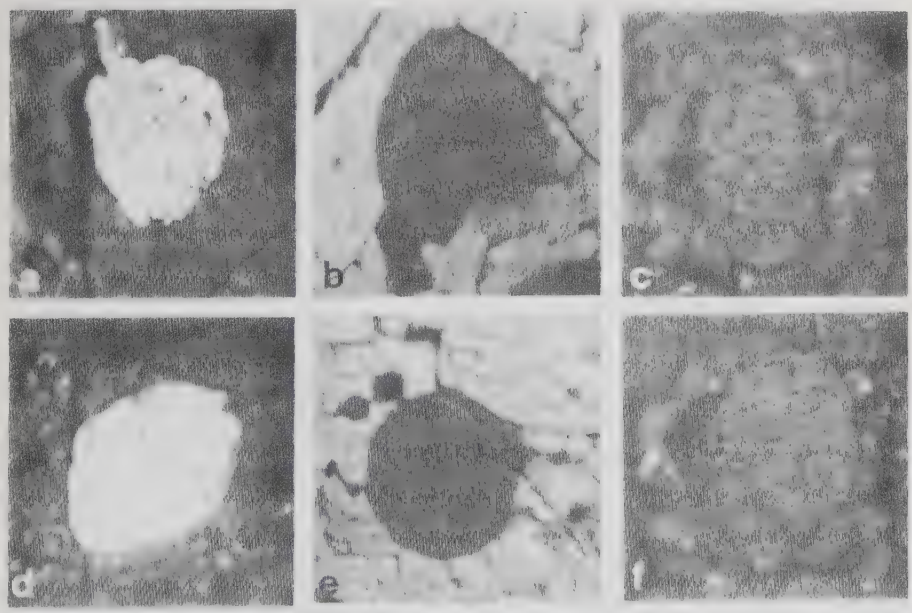


Figure 3. Formaldehyde-induced fluorescence in islet cells of monoamine oxidase inhibitor pretreated mice 2 h after injection of; a) L-DOPA b) Decarboxylase inhibitor + L-DOPA c) Dopamine d) L-5-HTP e) Decarboxylase inhibitor + L-5-HTP f) 5-HT. After decarboxylase inhibition (b and e) islets are devoid of fluorescence (dark in picture), while exocrine cells display fluorescence (X 150).

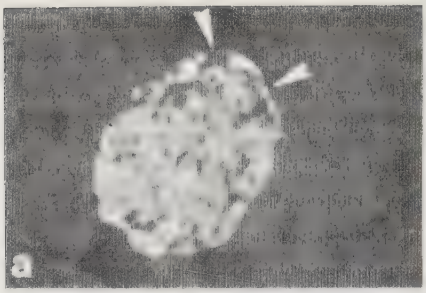


Figure 4. a) Formaldehyde-induced fluorescence in mouse pancreatic islet after monoamine oxidase inhibition + L-5-HTP. b) Same section processed for somatostatin immunoperoxidase. Non-fluorescent islet cells in a) (arrowheads) correspond with somatostatin cells in b) (X 200).

large dose of pargyline at -24 h in combination with the lower dose at -4 h and L-DOPA at -2 h, elicited a moderate hypoglycaemia not induced by insulin. Blood samples were drawn immediately before and 2 min after a rapid intravenous injection of half-maximal doses of the secretagogues, respectively. The doses were: L-IPNA 0.34 µmol/kg; carbachol 0.16 µmol/kg; arginine 1.2 mmol/kg. Previous experiments had shown that peak levels of both insulin and glucagon were reached about 2 min after the injection of all 3 secretagogues except that following L-IPNA insulin peaked at 5 min (22). Fig. 5 shows that dopamine accumulation (see Fig. 3a) at normal plasma glucose levels suppressed the insulin response induced by carbachol or L-IPNA, whereas that induced by arginine was unaffected. A similar pattern, but a greater suppression, was recorded during extended monoamine oxidase inhibition, which is accompanied by hypoglycaemia. Glucagon release at normal plasma glucose levels was suppressed by dopamine after L-IPNA but not after carbachol or arginine. Hypoglycaemia did not change this suppression of L-IPNA-stimulated glucagon release, but increased the glucagon response induced by carbachol or arginine (Fig. 5).

Influence of L-DOPA Induced Dopamine Accumulation on $^{45}Ca^{2+}$ Efflux in Perifused Rat Islets.
This series of experiments was initiated to test our hypothesis that intracellular dopamine may influence calcium translocation processes in the insulin cell (5). Fig. 6 (upper panel) shows that in the presence of the monoamine oxidase inhibitor pargyline L-DOPA (1.25 mM) induced a marked and immediate increase in the rate of $^{45}Ca^{2+}$ efflux. The fractional loss of $^{45}Ca^{2+}$ from the islets during the total L-DOPA infusion time was 18.6 ± 1.6% (Mean ± SEM) compared with 14.3 ± 0.6% for the control islets ($P<0.05$). L-DOPA-treated islets showed a slight tendency to a lower basal insulin secretion than the controls, significant at 70 min only ($P < 0.05$). Infusion of L-DOPA in the presence of the decarboxylase inhibitor benserazide, did not increase $^{45}Ca^{2+}$ efflux over control (Fig. 6) (lower panel), suggesting that the observed effect (upper panel) was not due to L-DOPA but rather to dopamine. No effect on basal insulin release in the presence of benserazide was observed.

DISCUSSION

There is much evidence that the pancreatic islet of most mammals are innervated by the autonomic nervous system which seems to be involved in the regulation of both insulin and glucagon secretion (16,23,32,35). We now report that one aspect of the nervous regulation of islet cell function in the guinea-pig is the modulation of the 5-HT content of the insulin cells. Thus vagotomy decreased and chemical sympathectomy increased the 5-HT content. This observation is suggestive of an interaction between the autonomic nervous system and the intracellular amines in the formation, storage and/or release of insulin. Since the 5-HT-store in the guinea-pig insulin cells seems to be of no

Table I. In vivo uptake of L-^{14}C-DOPA and ^{14}C-Dopamine in mouse islet tissue.

Minutes after injection	L-DOPA	Dopamine
	pmol equivalents/islet	
10	1.60	0.02
120	6.95	0.02

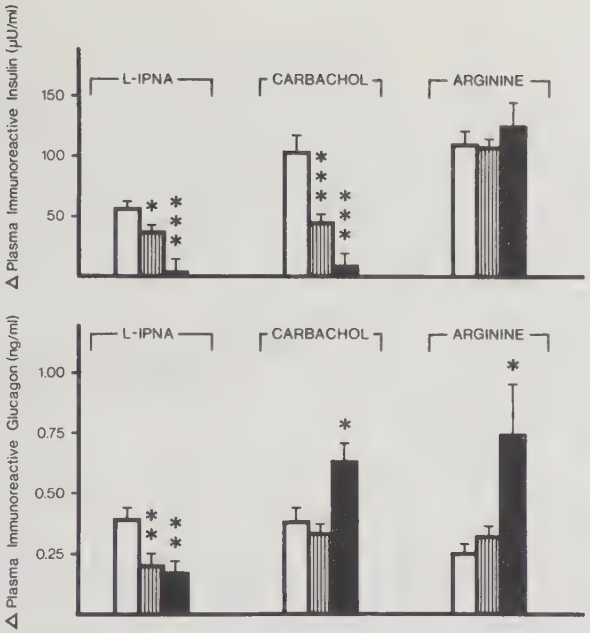


Figure 5. Increase in plasma levels of insulin and glucagon 2 min after i.v. injection of half-maximal doses of L-IPNA, carbachol, or arginine. Pretreatment with saline (open columns) (plasma glucose 6.6 ± 0.1 mmol/l); pargyline + L-DOPA (striped columns) (plasma glucose 6.7 ± 0.1 mmol/l); or 2 doses of pargyline + L-DOPA (black columns) (plasma glucose 2.6 ± 0.2 mmol/l). 14-26 mice in each group. Means ± SEM. Asterisks denote significant differences from saline pretreated controls, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

importance for insulin synthesis or storage (26,28) it is conceivable that the intracellular 5-HT concentration may influence basal or stimulated insulin secretion. It remains to be established whether exogenously-induced amine accumulation in the mouse insulin cells simulates the physiological situation in the guinea-pig insulin cells (Fig. 5)(5,21,22).

It should be emphasized again that neither 5-HT nor dopamine have been convincingly shown to be indigenous to the insulin and glucagon cells of the rat and mouse. In fact, arylethylamines are non-detectable by available histochemical methods in islet cells of several species. However, like most peptide hormone-producing cells insulin and glucagon cells in the mouse are notable for their capacity to take up and decarboxylate exogenous amine precursors. Among the different islet endocrine cell types (27), the somatostatin cells, however, are exceptional in that they seem incapable of taking up and decarboxylating L-DOPA and L-5-HTP. This is in agreement with earlier observations using electron microscopic autoradiography (4). One question which has been much debated (10,14,16,21, 22,29,33) is whether amines such as 5-HT and dopamine can gain entrance into the islet cells and act as intracellular granule-bound amines or whether they have to be taken up as the precursors to be decarboxylated and then stored in the cytoplasm. Although there seems to be a notable intracellular uptake of the amines under *in vitro* conditions (14, 29) our results show that the uptake of 5-HT or dopamine *in vivo* is extremely poor, indeed. The reason for this discrepancy remains to be elucidated. Thus, effects of extracellular amines on islet cell function *in vivo* are most probably brought about by actions on islet cell membrane receptors.

Our present *in vivo* experiments on the effect of intracellularly accumulated dopamine on insulin and glucagon secretion were performed according to the pretreatment schedule in Fig. 3a in one group of mice. Another group was subjected to an extended period of monoamine oxidase inhibition in order to achieve a hypoglycaemic condition 2 h after L-DOPA. This hypoglycaemia shows similarities to that induced by 5-HTP (22) and does not result from elevated insulin levels. Thus, it seems to be a useful model when studying the glucose dependency of insulin and glucagon secretagogues. With regard to the inhibition of insulin secretion by intracellularly accumulated dopamine, the present results

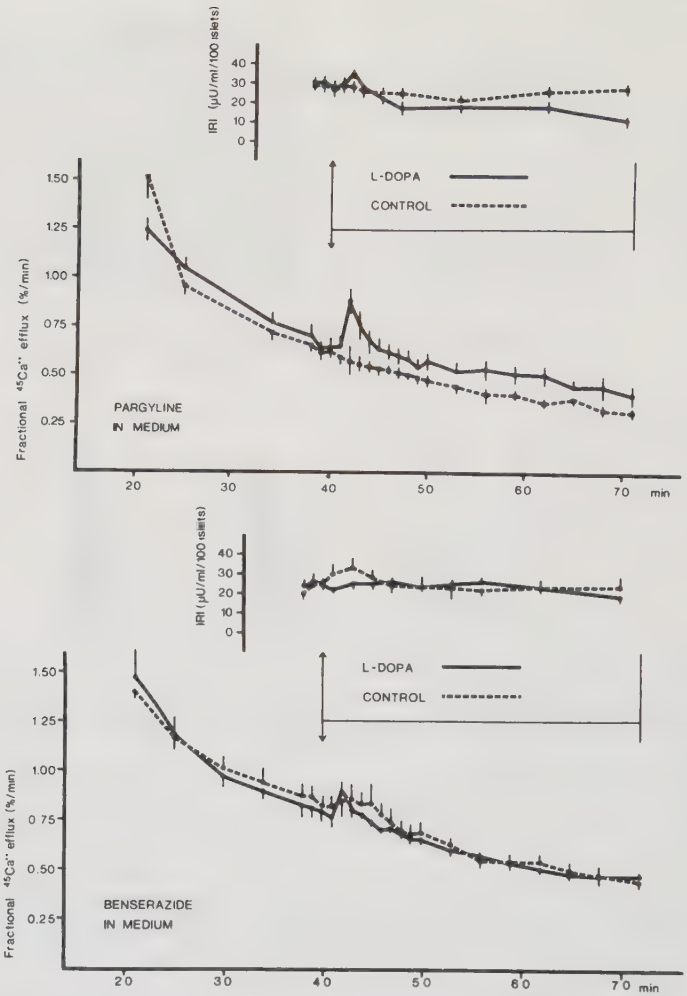


Figure 6. Effect of L-DOPA (1.25 mM) on insulin secretion and on efflux of $^{45}Ca^{2+}$ from prelabeled rat islets. Basal perifusate contained 100 μM pargyline (monoamine oxidase inhibitor) (upper panel), or 12.5 μM benserazide (decarboxylase inhibitor) (lower panel). Means ± SEM of 5 or 6 experiments in parallel chambers.

(Fig. 5) indicate that the insulin secretory pathway after cholinergic stimulation is affected most, the β-adrenergic is less affected and arginine-induced insulin release seems to be unaffected. Glucose-induced insulin release was previously found to be inhibited to a much smaller extent than that of L-IPNA (5). Hypoglycaemia plus extended monoamine oxidase inhibition further suppressed the insulin response to L-IPNA and carbachol, but did not influence insulin release after arginine. Intracellular accumulation of dopamine in the glucagon cells was found to suppress glucagon release induced by β-adrenergic stimulation, whereas release induced by carbachol and arginine seemed to be largely unaffected. Hypoglycaemia, which is known to enhance glucagon secretion, did increase the glucagon release after carbachol and arginine but did not influence the suppressive effect on the β-adrenergic pathway. Thus, intracellular accumulation of dopamine exerts its greatest suppressive effect on cholinergic insulin release and on β-adrenergic glucagon release. It is notable that these secretory pathways may be affected by direct nervous regulation as well as by other extracellular aminergic influences.

There is now good evidence that calcium is necessary for the release of both insulin and glucagon (8,9,19,24,25,34), although important quantitative and qualitative distinctions between insulin and glucagon cells may exist as to the exact mode of action of calcium after stimulation by different secretagogues. The results of our ^{45}calcium-efflux studies might give a clue to how the intracellular amines act. Our previous observations by ultrastructural autoradiography (5) suggested a location of dopamine to the peripheral part of the insulin secretory granules, outside the dense core which is known to contain insulin. A similar topographical localization of calcium, particularly after a secretory stimulus, has been demonstrated by analytical electron microscopy (15, 31). In accordance with these results recent biochemical observations in isolated islets indicate an increased deposition of $^{45}Ca^{2+}$ in the secretory granule fraction after stimulation with high glucose levels (2). To achieve such an increased deposition of $^{45}Ca^{2+}$ in the granules our islets were labeled with $^{45}Ca^{2+}$ in high glucose (16.7 mM) (7). Thus, it seems reasonable to assume that L-DOPA-induced dopamine accumulation in the secretory granules influenced the granule-pool of $^{45}Ca^{2+}$ to promote the observed increase in $^{45}Ca^{2+}$ efflux. This does not exclude that extracellular calcium or other cellular calcium pools were implicated. The interaction between amine and calcium is probably not a direct one, unless very specialized dopamine receptors are involved, but rather form part of a complex chain of reactions in the granules. The possibility of such a complex interaction is further underlined by the fact that insulin secretory granules have been shown to contain substantial amounts of adenine nucleotides (20) which are known to form aggregates with monoamines in other cell types and also to interact with divalent cations (1).

REFERENCES

1. Berneis, K.H., Pletsher, A. and DaPrada, M. (1969): Nature, 224, 281.
2. Bloom, G.D., Hellman, B., Sehlin, J. and Täljedal, I.-B. (1977): Amer. J. Physiol. 232, E114.
3. Cegrell, L. (1968): Acta physiol. scand. Suppl. 214, 1.
4. Ekholm, R., Ericson, L.E. and Lundquist I. (1971): Diabetologia 7, 339.
5. Ericson, L.E., Håkanson, R. and Lundquist, I. (1977): Diabetologia 13, 117.
6. Falck, B. and Hellman, B. (1963): Experientia 19, 139.
7. Frankel, B.J., Imagawa, W.T., O'Connor, M.D.L., Lundquist, I., Kromhout, J.A., Fanska, R. and Grodsky, G.M. (1978): J. Clin. Invest. 62, 525.
8. Gerich, J.E., Frankel. B.J., Fanska, R., West, L., Forsham, P.H. and Grodsky, G.M. (1974): Endocrinology 94, 1381.
9. Grodsky, G.M. and Bennett, L.L. (1966): Diabetes 15, 910.
10. Gylfe, E., Hellman, B., Sehlin, J. and Täljedal, I.-B. (1973): Endocrinology 93, 932.
11. Hansen, S.E. and Hedeskov, C.J. (1977): Acta endocrinol. (Kbh.): 86, 820.
12. Heding, L.G. (1966): In: Labelled Proteins in Tracer Studies, p. 345. Editors: L. Donato, G. Milhaud and J. Sirchis. Euratom, Brussels.
13. Heding, L.G. (1971): Diabetologia 7, 10.
14. Hellman, B., Lernmark, Å., Sehlin, J. and Täljedal, I.-B. (1972): Bioch. Pharm. 21, 695.
15. Herman, L., Sato, T. and Hales, L.N. (1973): J. Ultrastruc. Res. 42, 298.
16. Jacoby, J.H. and Bryce, G.F. (1978): General Pharmacol. 9, 411.
17. Jaim-Etcheverry, G. and Zieher, L.M. (1968): Endocrinology 83, 917.
18. Lebowitz, H.E. and Feldman, J.M. (1972): Fed. Proc. 32, 1797.
19. Leclerq-Meyer, V., Marchand, J. and Malaisse, W.J. (1978): Diabetes 27, 996.
20. Leitner, J.W., Sussman, K.E., Vatter, A.E. and Schneider, F.H. (1975): Endocrinology 96, 662.
21. Lundquist, I. (1971): Acta physiol. scand. Suppl. 372, 1.
22. Lundquist, I., Ekholm, R. and Ericson, L.E. (1971): Diabetologia 7, 414.
23. Lundquist, I. and Ericson, L.E. (1978): Cell Tiss. Res. 193, 73.
24. Lundquist, I., Fanska, R. and Grodsky, G.M. (1976): Endocrinology 98, 815.
25. Lundquist, I., Fanska, R. and Grodsky, G.M. (1976): Endocrinology 99, 1304.
26. Lundquist, I., Håkanson, R., Sundler, F. and Heding, L.G. (1978): Diabetologia 14, 251.
27. Lundquist, I., Sundler, F., Ahrén, B., Alumets, J. and Håkanson, R. (1979): Endocrinology 104, 832.
28. Lundquist, I., Sundler, F., Håkanson, R., Larsson, L.-I. and Heding, L.G. (1975): Endocrinology 97, 937.
29. Mahoney, C. and Feldman, J.M. (1977): Diabetes 26, 257.
30. Rerup, C. and Lundquist, I. (1966): Acta endocrinol. (Kbh.) 52, 357.
31. Schäfer, H.J. and Klöppel, G. (1974): Virchows Arch. (Pathol. Anat.) 362, 231.
32. Smith, P.H. and Porte, D. Jr. (1976): Ann. Rev. Pharm. Tox. 16, 269.
33. Sundler, F., Håkanson, R., Lorén, I. and Lundquist, I. (1979): Invest. Cell Pathol. (in press).
34. Wollheim, C.B., Blondel, B., Renold, A.E. and Sharp, G.W.G. (1976): Diabetologia 12, 287
35. Woods, S.C. and Porte, D. Jr. (1974): Phys. Rev. 54, 596.
36. Zern, R.T., Foster, L.B., Blalock, J.A. and Feldman, J.M. (1979): Diabetes 28,185.

Insulin Secretory Response to Different Secretagogues in Hyper- and Hypothyroid Mice

Bo Ahrén and Ingmar Lundquist

Department of Pharmacology, University of Lund, Lund, Sweden

ABSTRACT

The influence of long-term changes in thyroid state on insulin secretion was investigated *in vivo* in mice. Hyperthyroidism was induced by daily injections of L-triiodothyronine and hypothyroidism by a single injection of ^{131}I. Four different insulin secretagogues were used to characterize the insulin secretion, glucose, the β_2-adrenoceptor agonist terbutaline, the cholinergic agonist carbachol and the synthetic C-terminal octapeptide of cholecystokinin, CCK-8.

In the hyperthyroid mice the plasma glucose level was decreased to approximately 6 mmol/l compared to approximately 9 mmol/l in control mice. Despite this lower glucose level the insulin secretory responses to terbutaline and glucose were markedly potentiated (by about 200%). Insulin secretion in response to carbachol or CCK-8 was less enhanced (by about 50 and 100%, respectively). Liver and muscle glycogen levels were reduced by approximately 90% and 40%, respectively.

The hypothyroid animals showed blunted insulin secretory responses to all secretagogues, after terbutaline by 100%, after glucose by 50%, after carbachol by 70% and after CCK-8 by 50%. Plasma glucose and muscle glycogen levels were normal, whereas liver glycogen levels were enhanced by approximately 70%.

Thus, in the mouse, long-term hyperthyroidism is associated with a potentiated, and long-term hypothyroidism with a diminished insulin secretory response to four different secretagogues.

The β-adrenergically induced insulin release was most markedly affected by the thyroid state. The thyroid state may thus be of importance for the functional balance between α- and β-adrenoceptors. Since the thyroid activity was of importance for the response to all secretagogues it cannot be excluded that the thyroid state also exerts influences not related to the adrenoceptors on the secretory capacity of the insulin cell.

INTRODUCTION

The thyroid hormones are known to influence carbohydrate metabolism (Lam-

berg 1965). Thus, a decreased glucose tolerance is often noticed in patients with hyperthyroidism (Hales & Hyams, 1964; Doar et al. 1969; Nilsson et al. 1980). It has, furthermore, been demonstrated from studies in man that during hyperthyroidism β-adrenergically induced insulin secretion is enhanced (Wajchenberg et al. 1978), whereas the results concerning thyroid influences on glucose-induced insulin release are conflicting, suggesting enhanced, unchanged or diminished insulin secretory response to intravenously or orally administered glucose in hyperthyroidism (Hales & Hyams 1964; Doar et al. 1969; Andreani et al. 1970; Cavagnini et al. 1974; Andersen et al. 1977; Wajchenberg et al. 1978; Nilsson et al. 1980; Kabadi & Eisenstein 1980). In rats, pretreatment with daily injections of thyroxine resulted in an increased insulin secretion in response to β-adrenergic stimulation whereas hypothyroid rats had a decreased response (Okajima & Ui 1978). Moreover, glucose-induced insulin secretion has been shown to be enhanced after long-term hyperthyroidism *in vivo* in dogs (Renauld et al. 1978) and diminished after short-term hyperthyroidism *in vivo* in rats (Okajima & Ui 1978) and dogs (Renauld et al. 1971) as well as *in vitro* (rat) (Malaisse et al. 1967; Lenzen 1978). Diminished glucose-induced insulin secretion was seen *in vitro* in hypothyroid rats (Malaisse et al. 1967).

In the present study, the effects of long-term hyper- and hypothyroid states on the insulin secretory response to different secretagogues were investigated *in vivo* in the conscious mouse. Four different secretagogues were used, glucose, the β_2-adrenoceptor agonist terbutaline, the cholinergic agonist carbachol and the C-terminal octapeptide of cholecystokinin, CCK-8.

MATERIAL AND METHODS

Animals. Female mice of the NMRI strain (Laboratory Animal Breeding, Laven, Denmark) were used throughout the experiments. The animals were kept on a standard pellet diet (Astra-Ewos, Södertälje, Sweden), and tap water *ad libitum* before and during the experiments.

Drugs. L-triiodothyronine sodium (Sigma Chemical Company, St. Louis, Mo, USA) was dissolved in ethanol, 70%, with 0.1 N NaOH. D-glucose, carbachol (British Drug Houses Ltd., Poole, England) and DL-terbutaline sulfate (1-(3,5-dihydroxyphenyl)-2-(tert-butylamino)ethanol sulfate) (Draco AB, Lund, Sweden) were dissolved in saline, and synthetic CCK-8 (Kinevac®) (Squibb, New Brunnswick, N.J., USA) was dissolved in saline with addition of 0.1% gelatine to avoid adsorbtion. $Na^{131}I$ was from the Radiochemical Centre Ltd., Amersham, England.

Pretreatment. Hyperthyroidism was induced by daily subcutaneous injections of L-triiodothyronine, 5 µg/mouse, for three weeks before the experiments. Hypothyroidism was induced by a single i.p. injection of 200 µCi ^{131}I, and 3 weeks later the experiments were performed. Controls were treated with the solvent for L-triiodothyronine daily for 3 weeks, or with a single i.p. injection of saline 3 weeks before the experiments, respectively.

Experiments. D-glucose, 2.78 mmol/kg, carbachol, 0.16 µmol/kg, CCK-8, 6.25 nmol/kg, or terbutaline, 3.6 µmol/kg, was injected i.v. in a tail vein; 10 µl/g body weight was given. Blood samples were taken by orbital puncture 2 (D-glucose, carbachol or CCK-8) or 5.5 (terbutaline) minutes after the injection. In initial experiments it was found that the peak level of plasma concentrations of immunoreactive insulin is achieved about 2 (glucose, carbachol and CCK-8) or 5.5 (terbutaline) minutes after an i.v. injection and the time intervals to the peak levels were not altered after manipulations with the thyroid state. The doses were chosen to yield a comparable

increase in plasma concentrations of immunoreactive insulin. Controls were injected with saline, 10 µl/g body weight, and blood samples were taken 2 or 5.5 minutes later. There was no difference with regard to plasma immunoreactive insulin or glucose 2 or 5.5 minutes after an injection of saline, and thus all saline-injected animals are treated as a homogenous control group.

Determination of Insulin, Glucose and Glycogen. The plasma concentration of immunoreactive insulin (IRI) was determined by radioimmunoassay using ^{125}I-labelled pig insulin and guinea pig anti-pig insulin (Heding 1966), and plasma concentration of glucose was determined with a glucose oxidase method (Bruss & Black 1978). Glycogen content of liver and muscle (gastrocnemius) was measured according to Rerup & Lundquist 1967.

RESULTS

Body Weight. The weight gain during the 3 weeks of treatment was about the same in the hyperthyroid group as in the control group. The hypothyroid animals, however, did not gain any weight during the 3 weeks period that followed after radiothyroidectomy. The hypothyroid animals increased 0.3 $\pm$ 0.1 g during the three weeks after ^{131}I treatment and their controls 3.5 $\pm$ 0.2 g ($p < 0.001$).

Insulin Release in the Hyperthyroid State. Fig. 1 shows the plasma concentrations of IRI and glucose in the hyperthyroid animals and their controls in the basal state and after stimulating insulin secretion by the four secretagogues. It is seen that basal insulin levels were similar in controls and in triiodothyronine-pretreated animals, although the hyperthyroid animals had lower plasma glucose concentrations; 8.9 $\pm$ 0.2 mmol/l in the control group, and 6.1 $\pm$ 0.2 mmol/l in the thyrotoxic group ($p < 0.001$). The insulin secretory response was markedly enhanced in the hyperthyroid animals after injection of terbutaline and glucose, by about 200%, respectively, and moderately enhanced after treatment with carbachol and CCK-8, by about 50% and 100%, respectively. Thus, the hyperthyroid animals showed, despite the lower plasma glucose, an increased insulin secretion in response to all secretagogues.

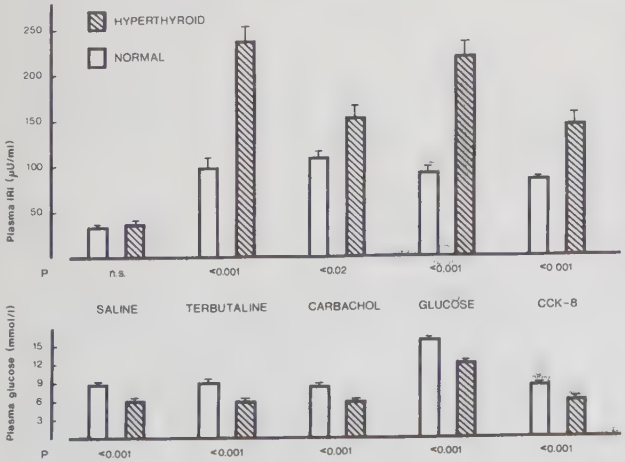


Fig. 1

Hyperthyroid animals. Plasma concentrations of immunoreactive insulin (peak level) and glucose after the i.v. injection of terbutaline 3.6 µmol/kg, carbachol 0.16 µmol/kg, glucose, 2.78 mmol/kg, or CCK-8 6.25 nmol/kg. Saline was injected in controls. Open columns: control animals. Striped columns: hyperthyroid animals. Each group consisted of 27-30 animals. P probability level of random difference. Bars indicate SEMs.

Determination of liver and muscle glycogen levels revealed that the hepatic glycogen stores were almost depleted in our hyperthyroid animals. Muscle

glycogen concentration was reduced by about 40% (Table 1).

Table 1

Liver and muscle (gastrocnemius) glycogen in normal and hyperthyroid mice (m ± s.e.m.).

	Liver glycogen mg/g wet weight	Muscle glycogen mg/g wet weight	
Hyperthyroid mice	6.3 ± 1.1	1.9 ± 0.2	n = 10
Control mice	61.2 ± 1.6	3.3 ± 0.3	n = 10
Probability level of random difference	p < 0.001	p < 0.01	

Insulin Release in the Hypothyroid State. Fig. 2 shows the plasma concentration of IRI and glucose in hypothyroid animals and their controls in the basal state and after stimulation by the different secretagogues. It is seen that basal insulin and glucose concentrations were normal in the hypothyroid animals, but the insulin secretory response was diminished in these animals to all secretagogues. The insulin response to terbutaline and carbachol, respectively, was totally and almost totally abolished, while the insulin secretory responses to glucose and to CCK-8 were decreased by about

Fig. 2

Hypothyroid animals. Plasma concentrations of immunoreactive insulin (peak level) and glucose after the i.v. injection of terbutaline, 3.6 µmol/kg, carbachol, 0.16 µmol/kg, glucose, 2.78 mmol/kg, or CCK-8, 6.25 nmol/kg. Saline was injected in controls. Open columns: normal animals. Striped columns: hypothyroid animals. Each group consisted of 22-30 animals. P probability level of random difference. Bars indicate SEMs.

50%. The hypothyroid state did not influence muscle glycogen levels, whereas liver glycogen concentration was markedly enhanced (Table 2).

Table 2

Liver and muscle (gastrocnemius) glycogen in normal and hypothyroid mice (m ± s.e.m.)

	Liver glycogen mg/g wet weight	Muscle glycogen mg/g wet weight	
Hypothyroid mice	72.7 ± 2.1	3.5 ± 0.3	n = 10
Control mice	42.2 ± 3.6	3.2 ± 0.3	n = 10
Probability level of random difference	p < 0.001	n.s.	

DISCUSSION

Insulin secretion is stimulated by several agents, and in this study four different secretagogues were selected in order to enhance the discharge of insulin. These were glucose, the cholinergic agonist carbachol, the β_2-adrenoceptor agonist terbutaline and the C-terminal octapeptide of cholecystokinin, CCK-8. It is not inconceivable that these secretagogues initiate different secretory mechanisms in the insulin cell, and evidence is accumulating that they use separate or partly separate secretory pathways to elicit the effects. In the present study the influence of the thyroid state on these secretory pathways was investigated.

Long-term hyperthyroidism in mice was associated with a markedly elevated insulin secretory response to all the studied secretagogues, whereas hypothyroid mice had a blunted response. Thus, the thyroid state seems to be of importance for insulin secretion after stimulation of these, at least partially different, secretory pathways. The responses to terbutaline and glucose were more potentiated in the hyperthyroid animals than those to CCK and carbachol, while the most depressed responses among the hypothyroid animals were seen after terbutaline and carbachol stimulation.

It should be noted that the plasma glucose was much reduced after treatment with triiodothyronine, and despite this the insulin secretory responses were notably enhanced. After hyperthyroidism in man elevated plasma glucose concentrations are often noted (Lamberg 1965; Andersen et al. 1977), but also normal values have been reported (Hales & Hyams 1964). The increased levels of plasma glucose with a concomitantly observed increased secretion of insulin suggests a decreased peripheral insulin sensitivity in hyperthyroidism (Andersen et al. 1977). After triiodothyronine treatment in mice, however, no change in the insulin sensitivity might evolve, and thus the increased responsiveness of the insulin cells leads to a decreased plasma glucose. In addition, the decreased plasma glucose after triiodothyronine treatment might be explained, at least partially, by exhaustion of the glycogen reserves (see also Lamberg 1965).

Earlier it has been shown that β-adrenergic stimulation of insulin secretion is enhanced in hyperthyroid rats (Okijama & Ui 1978). From *in vitro* studies on cardiac membranes an increased number of β-adrenergic receptors has been shown to evolve in response to thyroid hormone treatment (Banerjee & Kung 1977; Ciaraldi & Marinetti 1977), and similar findings have been demonstrated in fat cells (Giudicelli 1978). Others have shown, not an increased number of β-adrenergic receptors but an increased responsiveness of each receptor after thyroid hormone administration (Malbon et al. 1978; Bilezikian et al. 1979). Increased sensitivity of α-adrenergic receptors in hypothyroidism (Rosenqvist 1972; Grill & Rosenqvist 1973), strengthens the hypothesis that the thyroid hormones are of importance for the balance between α- and β-adrenergic receptors and the great enhancement of the insulin secretory response to terbutaline in hyperthyroid mice and the abolished response in hypothyroid mice might be explained by a change in this balance by the thyroid hormones. Also, the increase in the release of noradrenaline from sympathetic neurons in hypothyroidism and the reverse in hyperthyroidism (Spaulding & North 1975) may be of importance for the altered insulin secretion.

Glucose-induced insulin release *in vivo* has been shown to be enhanced in hyperthyroidism of long-term duration in man (Andersen et al. 1977; Kabadi & Eisenstein 1980) and dog (Renauld et al. 1978). However, also diminished insulin secretory response to glucose has been reported in man (Cavagnini et al. 1974). Furthermore, a diminished glucose effect on insulin secretion was noted in fasted rats (Okijama & Ui 1978) and

dogs (Renauld et al. 1971) and *in vitro* (rat) (Malaisse et al. 1967; Lenzen 1978) after short-term thyroxine administration. Species differences and different experimental procedures with special attention to food restriction and probably of most importance, the duration of the hyperthyroid state, might be reasons for the discrepancy against the results, and our long-term study doubtless shows an enhanced glucose-induced insulin response in hyperthyroid and a blunted response in hypothyroid mice. The connection between glucose and the ß-adrenoceptors with a potentiation of glucose-induced insulin release after stimulation of ß-adrenergic receptors may partly explain these findings (Wajchenberg et al. 1978).

The present study shows that synthetic CCK-8 stimulates insulin secretion in normal mice, and that also this secretory response was, as that to carbachol, enhanced after triiodothyronine-treatment and diminished after radiothyroidectomy.

It is thus striking that *in vivo* in mice all these secretagogues exerted insulin secretory responses with a gross potency in parallel with the long-term thyroid state. The influences of thyroid hormones on secretory events common for all these secretagogues seem thus to be a plausible explanation for the results. The thyroid hormones may additionally modulate some special events, such as adrenergic stimulation, and thus more markedly influence the secretory responses to agents most coupled to these receptors, e.g. terbutaline and perhaps glucose. Since cytosolic Ca^{++} may be of the utmost importance for insulin secretion it is of interest that thyroid hormones have been suggested to control certain Ca^{++}-pools in rat liver cells (Rosenqvist 1978). Enhanced translocation of Ca^{++} to stimulatory pools in hyperthyroid animals may explain increased insulin secretory responses to Ca^{++}-dependent secretagogues, such as glucose (Hellman et al. 1979) or carbachol (Burr et al. 1976). This does not exclude that other important effects of thyroid hormones, such as protein synthesis stimulation, effects on phosphodiesterase activity or changes in Na^{+}/K^{+} pump activity (for recent review see Sterling 1978), may add to the other effects of thyroid hormones after long term administration and change the insulin secretory response to all secretagogues.

ACKNOWLEDGEMENTS

The authors express their appreciation to Lena Kvist and Peter Okmark for technical assistance and to Eva Björkbom for secretarial help.

This study was supported by the Swedish Medical Research Council (04P-4289, 14X-4289) and by grants from the Medical Faculty, University of Lund, Lund, Sweden.

REFERENCES

Andersen O O, Friis T & Ottesen B (1977): Glucose tolerance and insulin secretion in hyperthyroidism. Acta Endocrinol (Kbh) 84:576-587,

Andreani D, Menzinger G, Fallucca F, Aliberti G, Tamburrano G & Cassano C (1970): Insulin levels in thyrotoxicosis and primary myxoedema: Response to intravenous glucose and glucagon. Diabetologia 6:1-7.

Banerjee S P & Kung L S (1977): ß-Adrenergic receptors in rat heart: Effects of thyroidectomy. Europ J Pharmacol 43:207-208.

Bruss M L & Black A L (1978): Enzymatic microdetermination of glycogen. Analyt Biochem 84:309-312.

Bilezikian J P, Loeb J N & Gammon D E (1979): The influence of hyperthyroidism and hypothyroidism in the β-adrenergic responsiveness of the turkey erythrocyte. J Clin Invest 63:184-192.
Burr I, Slonim A E, Burke V & Fletcher T (1976): Extracellular calcium and adrenergic and cholinergic effects on islet β-cell function. Am J Physiol 231:1246-1249.
Cavagnini F, Peracchi M, Raggi U, Bana R, Pontiroli A E, Malinverni A & Pinto M (1974): Impairment of growth hormone and insulin secretion in hyperthyroidism. Europ J Clin Invest 4:71-77.
Ciaraldi T & Marinetti G V (1977): Thyroxine and propylthiouracil effects in vivo in alpha and beta adrenergic receptors in rat heart. Biochem Biophys Res Commun 74:984-991.
Doar J W H, Stamp T C B, Wynn V & Audhya T K (1969): Effects of oral and intravenous glucose loading in thyrotoxicosis. Diabetes 18:633-639.
Giudicelli Y (1978): Thyroid-hormone modulation of the number of β-adrenergic receptors in rat fat-cell membranes. Biochem J 176:1007-1010.
Grill V & Rosenkvist U (1973): Inhibition of the noradrenaline-induced adenyl cyclase stimulation by augmented α-adrenergic response in subcutaneous adipose tissue from hypothyroid subjects. Acta Med Scand 194:129-133.
Hales C N & Hyams D E (1964): Plasma concentrations of glucose, non-esterified fatty acid, and insulin during oral glucose-tolerance tests in thyrotoxicosis. Lancet 2:69-70.
Heding L (1966): A simplified insulin radioimmunoassay method. In: Donato L, Milhaud G & Sirchis J: Labelled proteins in tracer studies. Euratom, Brussels, pp 345-350.
Hellman B, Andersson T, Berggren P O, Flatt P, Gylfe E & Kohnert K D (1979): The role of calcium in insulin secretion. In: Dumont J E & Nunez J: Hormones and cell regulation. Elsevier, Amsterdam, pp 69-97.
Kabadi U M & Eisenstein A B (1980): Glucose intolerance in hyperthyroidism: Role of glucagon. J Clin Endocrinol 50:392-396.
Lamberg B A (1965): Glucose metabolism in thyroid disease. Acta Med Scand 178:351-362.
Lenzen S (1978): Dose-response studies on the inhibitory effect of thyroid hormones on insulin secretion in the rat. Metabolism 27:81-88.
Malaisse W J, Malaisse-Lagae F & McGraw E F (1967): Effects of thyroid function upon insulin secretion. Diabetes 16:643-646.
Malbon C C, Moreno F J, Cabelli R J & Fain J N (1978): Fat cell adenylate cyclase and β-adrenergic receptors in altered thyroid states. J Biol Chem 253:671-678.
Nilsson O R, Kågedal B & Tegler L (1980): Insulin release and carbohydrate tolerance in hyperthyroid patients during non-selective or selective β_1-adrenoceptor blockade. Acta Endocrinol (Kbh) 93:179-185.
Okajima F & Ui M (1978): Adrenergic modulation of insulin secretion in vivo dependent on thyroid states. Am J Physiol 234:E106-111.
Renauld A, Pinto J E B, Sverdlik R C & Foglia V G (1971): Studies on the effect of hyperthyroidism on the insulin response to hyperglycemia in the dog. Horm Metab Res 3:247-251.
Renauld A, Sverdlik R C & Rodriquez R R (1978): Metabolic and endocrine effects of long-term thyroxine treatment. Horm Metab Res 10:568-569.
Rerup C & Lundquist I (1967): Non-specific reaction of current glucose oxidase preparations with glycogen and its application for glycogen determinations in tissue. Acta Pharmacol Toxicol 25:41-53.
Rosenqvist U (1972): Inhibition of noradrenaline-induced lipolysis in hypothyroid subjects by increased α-adrenergic responsiveness. Acta Med Scand 192:353-359.